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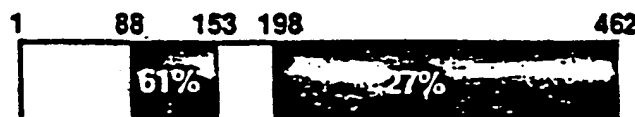
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(57) Abstract

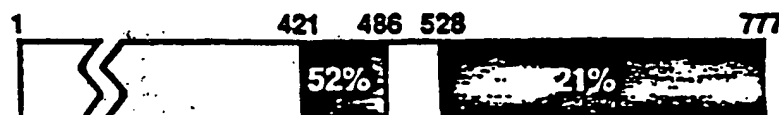
The present invention re-
lates to novel receptor polypep-
tides, which, upon interaction
with certain ligands, or activation
by certain compounds, modulate
transcription of certain genes by
binding to cognate response ele-
ments associated with promoters
of such genes. The novel recep-
tors of the invention modulate
transcription in the presence of
retinoid compounds. The recep-
tors of the present invention dif-
fer significantly from known reti-
noid acid receptors, in protein
primary sequence and in respon-
siveness to exposure to various
retinoids. The invention provides
DNAs encoding the novel recep-
tors, expression vectors for ex-
pression of the receptors, cells
transformed with such expres-
sion vectors, cells co-transformed
with such expression vectors and
with reporter vectors to monitor

mRXR α hRAR α 

hER

hTR β 

hGR



modulation of transcription by the receptors, and methods of using such co-transformed cells in screening for compounds which are capable, directly or indirectly, of activating the receptors. The invention also provides nucleic acid probes for identifying DNAs which encode additional retinoid receptors of the same class as the novel receptors disclosed herein.

RETINOID RECEPTOR COMPOSITIONS AND METHODS

RELATED APPLICATIONS

This application is a continuation-in-part of application Serial No. 478,071, filed February 9, 1990, now pending, the entire contents of which are hereby incorporated by reference herein.

5

TECHNICAL FIELD

The present invention concerns novel, steroid hormone-like receptor proteins and methods of making and using same.

10

More particularly, the invention relates to steroid hormone-like receptor proteins with transcription-modulating effects. Such proteins are responsive to the presence of retinoid acid and other vitamin A metabolites.

15

BACKGROUND OF THE INVENTION

The retinoids comprise a group of compounds including retinoid acid, retinol (vitamin A), and a series of natural and synthetic derivatives that together exert profound effects on development and differentiation in a wide variety of systems. Although early studies focused on the effects of retinoids on growth and differentiation of epithelial cells, their actions have been shown to be widespread. Many recent studies have examined the effects of these molecules on a variety of cultured neoplastic cell types, including the human promyelocytic leukemia cell line, HL60, where retinoid acid appears to be a potent inducer of granulocyte differentiation. In F9 embryonal carcinoma cells, retinoid acid will induce the differentiation of parietal endoderm, characteristic of a late mouse blastocyst. Retinoid acid also appears to play an important role in defining spatiotemporal axes in the developing avian limb and the regenerating amphibian limb.

Retinoid acid has been shown to induce the transcription of several cDNAs whose gene products have been isolated by differential screening. This observation supports the hypothesis that retinoid acid exerts its action via modulation of gene expression, in a manner analogous to the way in which steroid and thyroid hormones influence their target genes.

The ability to identify compounds which affect transcription of genes which are responsive to retinoid acid or other metabolites of vitamin A, would be of significant value, e.g., for therapeutic applications. Further, systems useful for monitoring solutions, body fluids and the like for the presence of retinoid acid, vitamin A or metabolites of the latter would be of value in various analytical biochemical applications and, potentially, medical diagnosis.

Through molecular cloning studies it has been possible to demonstrate that receptors for steroid, retinoid and thyroid hormones are all structurally related. These receptors comprise a superfamily of regulatory proteins that are capable of modulating specific gene expression in response to hormone stimulation by binding directly to cis-acting elements (Evans, Science 240, 889 (1988); Green and Chambon, Trends genet. 4, 309 (1988)). Structural comparisons and functional studies with mutant receptors have established that these molecules are composed of discrete functional domains, most notably, a DNA-binding domain that is composed typically of 66 - 68 amino acids (including two zinc fingers), and an associated carboxy terminal stretch of approximately 250 amino acids which comprises the ligand-binding domain (reviewed in Evans, supra).

Low-stringency hybridization has permitted the isolation and subsequent delineation of a growing list of gene products which possess the structural features of hormone receptors.

Recently, a retinoid acid dependent transcription factor, referred to as RAR-alpha (retinoid acid receptor-alpha), has been identified. Subsequently, two additional RAR-related genes have been isolated; thus there are now at least three different RAR subtypes (alpha, beta and gamma) known to exist in mice and humans. These retinoid acid receptors (RARs) share homology with the superfamily of steroid hormone and thyroid hormone receptors and have been shown to regulate specific gene expression by a similar ligand-dependent mechanism (Umesono et al., Nature 336, 262 (1988)). These RAR subtypes are expressed in distinct patterns throughout development and in the mature organism.

Other information helpful in the understanding and practice of the present invention can be found in commonly assigned, co-pending United States Patent Application Serial Nos. 108,471, filed October 20, 1987; 276,536, filed November 30, 1988; 325,240, filed March 17, 1989; 370,407, filed June 22, 1989; and 438,757, filed November 16, 1989, all of which are hereby incorporated herein by reference in their entirety.

SUMMARY OF THE INVENTION

We have discovered novel receptors which are activated to modulate transcription of certain genes in animal cells, when the cells are exposed to retinoids, such as retinoid acid and retinal. The novel receptors differ significantly from known retinoid acid receptors, both in terms of the primary protein sequence and responsiveness to various retinoids.

The novel receptors have several isoforms located at genetically distinct loci. They are capable of transactivating through cis elements similar to retinoid acid receptors, but show a different rank potency and dependency to retinoids. Northern analysis of the novel receptors of the present invention indicates that each isoform has a unique pattern of expression in adult

tissue and is temporally and spatially expressed in the embryo. Binding experiments demonstrate that the novel receptor proteins have a low affinity for [³H]retinoic acid. These results, taken together with results from transactivation studies, suggest the ligand(s) for the novel receptors is a metabolite(s) or structural analog(s) of retinoic acid. The invention provides DNAs encoding novel receptors, expression vectors for expression of the receptors, cells transformed with such expression vectors, cells co-transformed with such expression vectors and reporter vectors to monitor modulation of transcription by the receptors, and methods of using such co-transformed cells in screening for compounds which are capable, directly or indirectly, of activating the receptors.

The invention also provides single-stranded nucleic acid probes for identifying DNAs encoding additional retinoid receptors.

The invention also provides a method for making the receptors of the invention by expressing DNAs which encode the receptors in suitable host organisms.

Animal cells in which receptors of the invention are present can be employed to assay fluids for the presence of retinoids. Animal cells of the invention can also be employed to screen compounds of potential therapeutic value for their ability to bind and/or promote trans-activation (i.e., trans-acting transcriptional activation) by the receptors of the invention.

As will be described in greater detail below, the receptors of the invention modulate transcription of genes. This occurs upon binding of receptor to hormone response elements, which are positioned operatively, with respect to promoters for such genes, for such modulation to occur. Among hormone response elements contemplated for use in the practice of the present invention are TRE, the beta-retinoid acid response element, and the estrogen response element, as well as closely related elements

which are disclosed, for example, in Application Serial No. 438,757, filed November 16, 1989, and Application Serial No. 325,240, filed March 17, 1989.

5 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the extent of amino acid identity (i.e., "homology") between the DNA binding domain ("DNA") and ligand binding domain ("RX") of mouse RXR-alpha (mRXR α), relative to the corresponding domains of human
 10 retinoic acid receptor-alpha (hRAR α), human estrogen receptor (hER), human thyroid hormone receptor-beta (hTR β) and human glucocorticoid receptor (hGR).

Figure 2 shows the extent of amino acid identity (i.e., "homology") between the DNA binding domain ("DNA")
 15 and ligand binding domain ("LIGAND") of human RXR-alpha (hRAR α), relative to the corresponding domains of human retinoic acid receptor-beta (hRAR β), human retinoic acid receptor-gamma (hRAR γ), hTR β and hRXR α .

Figure 3 shows the extent of amino acid identity
 20 (i.e., "homology") between the DNA binding domain ("DNA") and ligand binding domain ("RX") of mRXR α , relative to the corresponding domains of mouse RXR-beta (mRXR β), mouse RXR-gamma (mRXR γ) and hRXR α .

Figure 4 illustrates the production of CAT from the
 25 reporter vector (ADH-TREp-CAT) in *Drosophila melanogaster* Schneider line 2 cells, which are co-transformed with receptor expression vector A5C-RXR-alpha and are in a medium containing various concentrations of retinoic acid.

30 Figure 5 illustrates the differences in transcription-activating activities of hRXR-alpha and hRAR-alpha, in mammalian cells in culture containing different vitamin A metabolites.

Figure 6, like Figure 5, illustrates the differences
 35 in transcription-activating activities of hRXR-alpha and hRAR-alpha in mammalian cells in culture containing retinoic acid or different synthetic retinoids.

Figure 7 illustrates the differences between hRXR-alpha and hRAR-alpha in dose-response to retinoic acid in media bathing mammalian cells in which the receptors occur. Figure 8 illustrates the differences between mouse RXR-alpha (mRXR α), mouse RXR-beta (mRXR β) and mouse RXR-gamma (mRXR γ) in dose response to retinoid acid (RA) in media bathing mammalian cells expressing such receptors.

Figure 9 illustrates the differences between mRXR α , mRXR β and mRXR γ in dose response to 3,4-didehydroretinoic acid (ddRA) in media bathing mammalian cells expressing such receptors.

DETAILED DESCRIPTION OF THE INVENTION

The invention concerns novel polypeptides, which are characterized by:

- (1) being responsive to the presence of retinoid(s) to regulate transcription of associated gene(s);
- (2) having a DNA binding domain of about 66 amino acids with 9 Cys residues, wherein said DNA binding domain has:
 - (a) less than about 65 % amino acid identity with the DNA binding domain of hRAR-alpha,
 - (b) less than about 55 % amino acid identity with the DNA binding domain of hRXR-beta, and
 - (c) less than about 55 % amino acid identity with the DNA binding domain of hGR; and
- (3) not including the sequence set forth in Sequence ID No 7.

The novel polypeptide receptors of the present invention can be further characterized in a variety of ways, e.g., by increasing the rate of transcription of a target gene in a construct comprising a promoter operatively linked to a human response element for

transcriptional activation by said receptors, relative to the rate of transcription in the absence of said receptors and/or in the absence of retinoic acid and retinal.

Transcription of said target gene is measured in an animal cell in culture, the medium of which comprises retinoid acid or retinal at a concentration greater than about 5×10^{-7} M.

Alternatively, the polypeptide receptors of the present invention can be further characterized as being encoded by a continuous nucleotide sequence which encodes substantially the same amino acid sequence as that of amino acids 1-462 shown in Sequence ID No. 2 [hRXR α], amino acids 1-467 shown in Sequence ID No. 4 [mRXR α], or amino acids 1-463 shown in Sequence ID No. 6 [mRXR γ].

As yet another alternative, the polypeptide receptors of the present invention can be characterized as being encoded by a continuous nucleotide sequence which encodes substantially the same amino acid sequence as that of amino acids 135-200 shown in Sequence ID No. 2 [DNA binding domain of hRXR α], amino acids 140-205 shown in Sequence ID No. 4 [DNA binding domain of mRXR α], or amino acids 139-204 shown in Sequence ID No. 6 [DNA binding domain of mRXR γ].

As still another alternative, the polypeptide receptor of the present invention can be characterized as being encoded by a continuous nucleotide sequence which is substantially the same as nucleotides 76-1464 shown in Sequence ID No. 1 [hRXR α], nucleotides 181-1581 shown in Sequence ID No. 3 [mRXR α], or nucleotides 123-1514 shown in Sequence ID No. 3 [mRXR γ].

As employed herein, the term "retinoids" refers to naturally occurring compounds with vitamin A activity synthetic analogs and various metabolites thereof. The retinoids are a class of compounds consisting of four isoprenoid units joined in head-tail manner.

Numerous retinoids have been identified, as described, for example, by Sporn, Roberts and Goodman in

th tw volum treatise entitled The Retinoids (Academic Press, NY, 1984), to which the reader is directed for further detail. Exemplary retinoids include retinol, retinyl acetate, retinyl hexadecanoate, α -retinyl, 4,14-retroretinol, deoxyretinol, anhydroretinol, 3,4-didehydroretinol, 15,15-dimethyl retinol, retinyl methyl ether, retinyl phosphate, mannosyl retinyl phosphate, retinol thioacetate, retinal (retinaldehyde), 3,4-didehydroretinal, retinylidene acetylacetone, retinylidene-1,3-cyclopentanedione, retinal oxime, retinaldehyde acetylhydrazone, retinoic acid, 4-hydroxyretinoic acid, 4-oxoretinoic acid, 5,6-dihydroretinoic acid, 5,6-epoxyretinoic acid, 5,8-epoxyretinoic acid, the open-chain C_{20} analog of retinoid acid (i.e., (all-E-3,7,11,15-tetramethyl-2,4,6,8,10, 2,14-hexadecaheptaenoic acid), 7,8-didehydroretinoic acid, 7,8-dihydroretinoic acid, " C_{15} Acid" (E, E)-3-methyl-5-(2,6,6-trimethyl-2-cyclohexen-1-yl)-2,4-pentanedioic acid), " C_{17} Acid" ((E,E,E)-5-methyl-7-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6-heptatrienoic acid), " C_{22} Acid" (14'-apo- β , ψ -carotenoic acid), retinoic acid esters (e.g., methyl ester, ethyl ester, etc.), retinoid acid ethylamide, retinoic acid 2-hydroxyethylamide, methyl retinone, " C_{18} Ketone" ((E,E,E)-6-methyl-8-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3,5,7-octatrien-2-one), and the like.

In addition, according to the present invention, there are provided DNA sequences which encode novel polypeptides as described above.

Further in accordance with the present invention, there are provided DNA constructs which are operative in animal cells in culture to make said polypeptides.

According to a still further embodiment of the present invention, there are provided animal cells in culture which are transfected with DNA constructs (as described above), which are operative in said cells to

make receptor polypeptides, by expression of DNA segments which encode the above described polypeptides.

Among the animal cells contemplated for use in the practice of the present invention are those which are further transformed with a reporter vector which comprises:

- (a) a promoter that is operable in the cell,
 - (b) a hormone response element, and
 - (c) a DNA segment encoding a reporter protein,
- wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and wherein said hormone response element is operatively linked to said promoter for activation thereof.

In accordance with the present invention, there is also provided a method of testing a compound for its ability to regulate the transcription-activating properties of the above-described receptor polypeptides, which method comprises assaying for the presence or absence of reporter protein upon contacting of cells containing a reporter vector and receptor polypeptide with said compound; wherein said reporter vector and said receptor polypeptide are as described above.

In accordance with a still further embodiment of the present invention, there are provided various probes, which can be used to identify genes encoding receptors related to those of the present invention. In this regard, particular reference is made to Examples V and VI below. More particularly, the invention provides labeled, single-stranded nucleic acids comprising sequences of at least 20 contiguous bases having substantially the same sequence as any 20 or more contiguous bases selected from:

- (i) bases 2 - 1861, inclusive, of the DNA illustrated in Sequence ID No. 1 [hRXR- α],

(ii) bas s 20 - 2095, inclusive, of the DNA
illustrated in S quenc ID No. 2 [mRXR- α],
or

(iii) bases 15 - 1653, inclusiv , f the DNA
illustrated in Sequence ID No. 3 [mRXR- γ],
or

(iv) the complement of any one of the sequences
according to (i), (ii), or (iii).

As employed herein, the term "labeled single-
stranded nucleic acid sequences" refers to single-
stranded DNA or RNA sequences which have been modified by
the addition thereto of a species which renders the
"labeled" sequence readily detectable from among other
unmodified sequences. Exemplary labels include
radioactive label (e.g., ^{32}P , ^{35}S), enzymatic label (e.g.,
biotin), and the like.

Preferred probes contemplated for use in the
practice of the present invention are those having at
least about 100 contiguous bases selected from the above-
described sequences. Especially preferred are probes
having in the range of about 198 up to several hundred
nucleotides, because greater selectivity is afforded by
longer sequences.

The invention also encompasses a method of making
the above-described receptor polypeptides, which method
comprises culturing suitable host cells which are
transformed with an expression vector operable in said
cells to express DNA which encodes receptor polypeptide.
Suitable hosts contemplated for use in the practice of
the present invention include yeast, bacteria, mammalian
cells, insect cells, and the like. *E. coli* is the
presently preferred bacterial species. Any of a number
of expression vectors are well known to those skilled in
th art that c uld b mployed in th method f th
inventi n. Among th se ar th prokaryotic expr ssi n
v ct rs pNH8A, pNH16A and pNH18A availabl from
Stratagene, La Jolla, Calif rnia USA.

Further information on the invention is provided in the following non-limiting examples and description of an exemplary embodiment.

5 EXAMPLES

Example I

The KpnI/SacI restriction fragment (503bp) including the DNA-binding domain of hRAR-alpha-encoding DNA [See Giguere et al., Nature 330, 624 (1987); and commonly
 10 assigned United States Patent Application Serial No. 276,536, filed November 30, 1988; and European Patent Application Publication No. 0 325 849, all incorporated herein by reference] was nick-translated and used to screen a lambda-gt11 human liver cDNA library
 15 (Kwok et al., Biochem. 24, 556 (1985)) at low stringency. The hybridization mixture contained 35% formamide, 1X Denhardt's, 5X SSPE (1X SSPE=0.15 M NaCl, 10mM Na₂HPO₄, 1mM EDTA), 0.1% SDS, 10% dextran sulfate, 100 mg/ml denatured salmon sperm DNA and 10⁶ cpm of [³²P]-labelled probe.
 20 Duplicate nitrocellulose filters were hybridized for 16h at 42°C, washed once at 25°C for 15 min with 2X SSC (1X SSC=0.15 M NaCl, 0.015 M sodium citrate), 0.1% SDS and then washed twice at 55°C for 30 min. in 2X SSC, 0.1% SDS. The filters were autoradiographed for 3 days at -70°C
 25 using an intensifying screen.

Positive clones were isolated, subcloned into pGEM vectors (Promega, Madison, Wisconsin, USA), restriction mapped, and re-subcloned in various sized restriction fragments into M13mp18 and M13mp19 sequencing vectors.
 30 DNA sequence was determined by the dideoxy method with Sequenase[™] sequencing kit (United States Biochemical, Cleveland, Ohio, USA) and analyzed by University of Wisconsin Genetics Computer Group programs (Devereux et al., Nucl. Acids Res. 12, 387 (1984)). A unique
 35 receptor-like sequence was identified and designated lambda-HL3-1.

Lambda-HL3-1 was used as a hybridization probe to rescreen a lambda-gt10 human kidney cDNA library (Arriza et al., Science 237, 268 (1987)) which produced several clones, the longest of which was sequenced and designated
5 lambda-XR3-1. The DNA sequence obtained as an EcoRI-fragment from lambda-XR3-1 has the sequence indicated in Sequence ID No. 1 [hRXR α].

Similar screening of a mouse whole embryo library with the full-length hRXR-alpha clone described above
10 provided additional sequences which encode different isoforms of the human RXR-alpha receptor. In addition, the mouse homolog (mouse RXR-alpha) was also identified in this way.

Thus, mRNA was isolated from 14.5 day post-coitus
15 (p.c.) mouse embryos, translated into cDNA, linked with EcoRI/NotI linkers, then inserted into the unique EcoRI site of the cloning vector λ -ZAP (Stratogene). The resulting library was screened at reduced stringency with ³²P-labeled, full length hRXR-alpha as the probe.

20 The DNA sequences of the resulting clones are set forth as Sequence ID No. 3 [mRXR α] and Sequence ID No. 5 [mRXR γ].

Example II

25 Amino acid sequences of mRXR-alpha, hRAR-alpha (human retinoic acid receptor-alpha), hER (human estrogen receptor) hTR-beta (human thyroid hormone receptor-beta) and hGR (human glucocorticoid receptor) were aligned using the University of Wisconsin Genetics Computer Group
30 program "Bestfit" (Devereux et al., supra). Regions of significant similarity between mRXR-alpha and the other receptors, i.e., the 66 - 68 amino acid DNA binding domains and the ligand-binding domains, are presented schematically in Figure 1 as percent amino acid identity.

35 Similarly, the amino acid sequences of human RAR-alpha (hRAR α), human RAR-beta (hRAR β), human RAR-gamma (hRAR γ), human TR-beta (hTR β) and human RXR-alpha (hRXR α)

were aligned. As done in Figure 1, regions of significant similarity between hRAR-alpha and the other receptors are presented schematically in Figure 2 as percent amino acid identity.

5 A further comparison of receptors is set forth in Figure 3. Thus, the amino acid sequences of mouse RXR-alpha (mRXR α), mouse RXR-beta (mRXR β), mouse RXR-gamma (mRXR γ) and human RXR-alpha (hRXR α) were aligned, and the percent amino acid identity presented schematically in
10 Figure 3.

Although the DNA-binding domains of both mRXR-alpha and hRXR-alpha are conserved relatively well with respect to other receptors (such as hRAR-alpha and hTR-beta), the ligand binding domain is poorly conserved. (See Figures
15 1 and 3). A comparison between the retinoic acid receptor subfamily of receptors and hRXR-alpha reveals nothing to suggest that hRXR-alpha is related to any of the known retinoid receptors (Fig. 2).

20 Example III

Drosophila melanogaster Schneider line 2 ("S2") cells (Schneider, *Embryol. Exp. Morphol.* 27, 353 (1972), which are readily available, were seeded at 2×10^6 per 35 mm² culture dish and maintained in Schneider medium
25 (GIBCO/Life Technologies, Inc., Grand Island, New York, USA) supplemented with penicillin, streptomycin and 12% heat-inactivated fetal bovine serum (Irvine Scientific, Santa Ana, California, USA). The cells were transiently co-transfected with 10 μ g/dish of plasmid DNA by calcium
30 phosphate precipitation (Krasnow et al., *Cell* 57, 1031 (1989): 4.5 μ g/dish of receptor expression vector or control construct (producing no hRXR-alpha); 0.5 μ g/dish of reporter plasmid or control reporter plasmid; 0.5 μ g/dish of reference plasmid; and 4.5 μ g inert plasmid
35 DNA.

In the receptor expression vector, A5C-RXR-alpha (4.5 μ g/dish), receptor hRXR-alpha is constitutively

expr ss d in the S2 cells under the control of the
Drosophila actin 5C promoter (A5C; Thummel et al., Gene
74: 445 (1988)) driving transcription of the EcoRI-site-
bound d ins rt of lambda-XR3-1. In th control vector,
5 A5C-RXR_{rev} (also 4.5 µg/ml), the EcoRI-site-bounded insert
from lambda-XR3-1 is inserted in the reverse (i.e., non-
coding or non-sense-coding) orientation.

A5C-RXR-alpha was made by first inserting at the
unique BamHI site of A5C a linker of sequence:

10

5'-GATCCGATATCCATATGGAATTCGGTACCA,

and then inserting, at the EcoRI site of the linker
(underlined above), the EcoRI-site-bounded insert of
15 lambda-XR3-1 (See Example I).

The reporter plasmid ADH-TRE_p-CAT (at 0.5 µg/dish)
contains the palindromic thyroid hormone response element
TRE_p, having the sequence:

20

5'-AGGTCATGACCT

[(Glass et al. Cell 54, 313 (1988); Thompson and Evans,
Proc. Natl. Acad. Sci. (USA) 86, 3494 (1989)], inserted
into position -33 (with respect to the transcription start
25 site) of a pD33-ADH-CAT background (Krasnow et al., Cell
57, 1031 (1989)).

pD33-ADH-CAT is a plasmid with the distal promoter of
the Drosophila melanogaster alcohol dehydrogenase gene
linked operably for transcription to the bacterial (E.
30 coli) chloramphenicol acetyltransferase ("CAT") gene, a
gene for the indicator protein CAT. ADH-TRE_p-CAT was made
by inserting the oligonucleotide of sequence:

35

5'-CTAGAGGTCATGACCT
TCCAGTACTGGAGATC-5'

into the XbaI site at position -33 in pD33-ADH-CAT. pD33-ADH-CAT, without TREp, served as a control reporter (i.e., background) plasmid.

A reference plasmid encoding beta-galactosidase driven by the actin 5C promoter also was transfected (0.5 μ g/dish) along with pGEM DNA (4.5 μ g/dish) (Promega, Madison, Wisconsin) to make up the final DNA concentration to 10 μ g/dish. The reference plasmid was made by inserting a BamHI-site bounded, beta-galactosidase-encoding segment into the unique BamHI site of A5C. The purpose of the reference plasmid was to normalize results for transfection efficiency.

Twenty-four hours post-transfection, various retinoids were added to the cultures. The retinoids were dissolved in dimethyl-sulfoxide and/or ethanol and the resulting solution was added to 0.1 % v/v of culture medium. Initial concentration of the retinoids in the culture media was 10^{-6} M, except for the experiments underlying the data displayed in Figure 4, for which varying concentrations of retinoic acid were used.

In control runs, ethanol, at 0.1 % v/v in the medium, was used in place of a solution of retinoid.

Cultures were maintained in the dark for 36 hr after addition of retinoid and then harvested. All other parts of the experiments, involving retinoids, were carried out in subdued light.

Cell lysates were centrifuged. Supernatants were assayed for beta-galactosidase, following Herbolme et al., Cell 39, 653-662 (1984), and units/ml of beta-galactosidase activity was calculated. CAT assays (normalized to beta-galactosidase activity) of supernatants were incubated for 75 unit-hours ("units" referring to units of beta-galactosidase activity), as described by Gorman et al., Mol. Cell. Biol. 2, 1044 (1982), usually 150 units for 30 minutes.

No hRXR-alpha dependent activation of CAT expression was noted in any experiment in which control reporter was

used in place of ADH-TREp-CAT. Similarly, essentially no activation was observed for runs where control plasmid, A5C-hRXR_{rev}, was used in place of A5C-hRXR.

The induction of CAT activity in retinoid-treated cells was compared with induction in untreated (i.e., only ethanol-treated) cells. Induction was measured in the presence of retinoic acid (RA), retinal (RAL), retinol acetate (RAC), retinol (ROH), and retinol palmitate (RP). The production of chloramphenicol acetyltransferase (CAT) from the reporter vector (ADH-TREp-CAT) was measured in *Drosophila melanogaster* Schneider line 2 cells, co-transformed with the hRXR-alpha expression vector A5C-RXR-alpha, and exposed to a medium to which retinoic acid (RA), retinal (RAL), retinol acetate (RAC), retinol (ROH), or retinol palmitate (RP) has been added to a concentration of 10^{-6} M. The relative induction observed was RA > RAL > RAC > ROH > RP.

In Figure 4 are displayed the results, also expressed in terms of "fold-induction" of CAT activity, as described in the previous paragraph, with retinoic acid at a number of different concentrations, to show the "dose response" of hRXR-alpha (in trans-activation at TREp in insect cells) to retinoid acid in the medium of the cells.

25

Example IV

This example, describing experiments similar to those described in Example III, shows that hRAR-alpha and hRXR-alpha differ significantly in their properties, specifically with respect to trans-activation of transcription from promoters.

The mammalian receptor-expression vector RS-hRAR-alpha, from which hRAR-alpha is produced under control of the 5'-LTR promoter of the rous sarcoma virus proviral DNA, is described in Giguere et al., *Nature* 330, 624 (1987); commonly assigned United States Patent Application Serial No. 276,536, filed November 30, 1988; and European

Patent Application Publication No. 0 325 849, all incorporated herein by reference.

Th receptor-expression vector RS-hRXR-alpha is constructed similarly to RS-hRAR-alpha, by inserting the
 5 EcoRI-site-bounded, hRXR-alpha-encoding segment of lambda-XR3-1 into plasmid pRS (Giguere et al., Cell 46, 645 (1986)).

Control plasmid pRSns is pRS with a non-sense-coding sequence inserted in place of receptor-coding sequence.

10 Reporter plasmid delta-MTV-TREp-CAT, also known as TREp1MCAT, has also been described (Umesono et al., Nature 336, 262 (1988), Thompson and Evans, supra., see also Umesono and Evans, Cell 57, 1139 (1989). When a control reporter, designated delta-MTV-CAT, which is substantially
 15 delta-MTV-TREp-CAT with TREp removed, was used in place of delta-MTV-TREp-CAT, no CAT activity was found with either receptor with any of the retinoids or retinoid analogs.

Reference plasmid, RS-beta-galactosidase, is also known and is substantially the same as RS-hRAR-alpha and
 20 RS-hRXR-alpha but has a beta-galactosidase-encoding segment in place of the receptor-encoding segment.

Culture of CV-1 cells, co-transfections (with reporter plasmid, receptor-expression-plasmid or control plasmid, reference plasmid and inert plasmid DNA) and CAT
 25 assays were performed as described in Umesono et al., Nature 336, 262 (1988). Co-transfections and CAT assays were carried out by methods similar to those described in Example III. Similar to the experiments in Example III, subdued light was used.

30 When CV-1 cells co-transformed with reporter plasmid (delta-MTV-TREp-CAT), reference plasmid, control plasmid (i.e., expressing no receptor), and receptor plasmid (RS-hRAR-alpha or RS-hRXR-alpha), were exposed to retinoids RA, RAL, RAC, ROH, RP, (which are naturally
 35 occurring vitamin A metabolites), or retinoid-free ethanol, the results shown in Figure 5 were obtained. The Figure illustrates production of CAT from reporter plasmid

in monkey kidney cells of the CV-1 line, which are co-transformed with hRXR-alpha-producing expression vector RS-hRXR-alpha or hRAR-alpha-producing expression vector RS-hRAR. Experiments are carried out in a medium to which
5 RA, RAL, RAC, ROH, or RP has been added to a concentration of 10^{-6} M. The bars over the "-" sign indicate the levels of CAT production when the cells are exposed to no retinoid (i.e., retinoid-free ethanol). The hatched bars indicate the level of CAT production when a control
10 expression vector, from which no receptor is expressed, is employed in place of the receptor expression vector. The open bars indicate the level of CAT production when receptor-producing expression vector is employed. In each case, the retinoids were added as ethanolic
15 solutions, with the volume of solution 0.1 % (v/v) in the medium. Retinoid-free ethanol was added to 0.1 % v/v. Results are plotted as percentages of the maximal response observed in the experiments, i.e., hRXR-alpha with RA.

In Figure 6, the results are provided for experiments
20 carried out as described in the previous paragraph but with, in place of RAL, RAC, ROH and RP, the synthetic retinoids 4-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-4-iodo-2-anthracyenyl)-benzoic acid ("R1"), ethyl-P-[(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)-1-
25 propenyl]-benzoic acid ("R2"), ethyl-all trans-9-(4-methoxy-2,3,6-trimethyl)-3,7-dimethyl-2,4,6,8-nonatetranoate ("R3"), and ethyl-all trans-9-(4-methoxy-2,3,6-trimethyl)-3,7-dimethyl-2,4,6,8-nonatetraenoic acid ("R4") initially at a concentration of 10^{-6} M. The Figure
30 illustrates production of CAT from the reporter plasmid (delta-MTV-TREp-CAT), CV-1 cells, which are co-transformed with hRXR-alpha-producing expression vector RS-hRXR-alpha or the constitutive hRAR-alpha-producing expression vector RS-hRAR. Experiments are carried out in a medium to which
35 RA, R1, R2, R3, or R4 has been added to a concentration of 10^{-6} M. The bars over the "-" sign indicate the levels of CAT production when the cells are exposed to no retinoid.

The hatched bars indicate the level of CAT production when a control expression vector, from which no receptor is expressed, is employed in place of the receptor expression vector. The open bars indicate the level of CAT production when receptor-producing expression vector is employed.

In Figure 7, results are presented for experiments carried out as described in this Example using various concentrations of retinoic acid. The Figure illustrates production of CAT from the reporter plasmid (delta-MTV-TRE_p-CAT), in CV-1 cells, which are co-transformed with the receptor-producing expression vector RS-RXR-alpha or RS-RAR-alpha. Experiments are carried out in a medium to which RA has been added to various concentrations. In the Figure, the results are in terms of fold-induction observed with cells exposed to RA, and control cells (exposed to only RA-free ethanol).

In Figure 8, results are presented for experiments carried out as described above, using various concentrations of retinoic acid with expression vectors encoding mRXR-alpha, mRXR-beta and mRXR-gamma.

In Figure 9, results are presented for experiments carried out as described above, using various concentrations of 3, 4-didehydroretinoic acid (ddRA) with expression vectors encoding mRXR-alpha, mRXR-beta and mRXR-gamma.

Example V

To determine the distribution of hRXR-alpha gene expression, poly A⁺ RNAs isolated from a variety of adult rat tissues were size fractionated, transferred to a nylon filter, and hybridized with hRXR-alpha cDNA.

Thus, for each tissue of adult male rat that was analyzed, total RNA was prepared from the tissue (see Chomczynski and Sacchi, Anal. Biochem. 162, 156 (1987)) and poly A⁺ selected by oligo(dT)-cellulose chromatography. Ten micrograms of poly A⁺ RNA were separated by 1% agarose-

formaldehyd g l lectrophoresis, transf rred to a Nytran filter (Schleicher and Schuell) (see McDonnell et al., Sci nc 235, 1214 (1987)), and hybridized under string nt conditions with the hRXR-alpha-encoding, EcoRI-insert of
5 lambda-XR3-1. Hybridization was performed at 42°C in a buffer containing 50% formamide, 5X Denhardt's, 5X SSPE, 0.1% SDS, 100mg/ml salmon sperm DNA, 200mg/ml yeast RNA, and [³²P]-labelled probe. The filter was then washed twice with 2X SSC, 0.1% SDS at 22°C and twice at 50°C.
10 Autoradiography was for 24h at -70°C with an intensifying screen. RNA ladder size markers from Bethesda Research Laboratories (Gaithersburg, Maryland, USA)

The distribution of RXR-alpha mRNA in the rat reveals a pattern of expression distinct from that of the retinoid
15 acid receptors (Giguere et al., Nature 330, 624 (1987); Zelent et al., Nature 339, 714 (1989); Benbrook, Nature 333, 669 (1988)). The rat RXR-alpha message appears to be a single species of about 4.8 kbp (kilobase pairs) which is expressed in many tissues, but most abundantly in the
20 liver, muscle, lung, and kidney and somewhat less abundantly in adrenal, heart, intestine, and spleen.

Example VI

Molecular cloning analyses of the thyroid hormone and
25 retinoic acid receptor genes indicate that each of these receptors belongs to a discreet gene subfamily which encode several receptor isoforms. To determine if this was also true of RXR, a series of Southern blot analyses were carried out. High stringency hybridization of
30 restriction endonuclease-digested human DNA with a labelled DNA fragment derived from lambda-XR3-1 produced a similar number of bands in every digestion, consistent with a single genetic locus. When the hybridization conditi ns were r lax d, howev r, many additional bands
35 w r bs rv d in th products of each nzyme digestion. Car ful inspecti n of this hybridizati n pattern demonstrat d that it is unrelat d to a similar analysis

described for hRAR-alpha (Giguere et al., Nature 330, 624 (1987). These observations indicate the presence of at least one other locus in the human genome related to the hRXR-alpha gene. Further, a genomic DNA zooblot representing mammalian, avian, yeast, and *Drosophila* species was obtained. Thus far, the RXR gene family appears to be present in all species tested except yeast, which to date has not been shown to contain any members of the steroid receptor superfamily.

For the analyses of human DNA, two human placenta genomic DNA Southern blots were prepared in parallel with identical DNA samples. The blots were hybridized at high or low stringency with a 1200 bp [³²P]-labelled fragment of lambda-XR3-1 which included the coding portions of the DNA and ligand binding domains (Sequence ID No. 1, nucleotides 459-1631).

For the zooblot, genomic DNA from human, monkey, rat, mouse, dog, cow, rabbit, chicken, *S. cerevisiae* and *Drosophila melanogaster* were hybridized at low stringency with a 330 bp [³²P]-labelled fragment of lambda-XR3-1 which included the DNA-binding domain (Sequence ID No. 1, nucleotides 459-776). Differently sized bands (in comparison with HindIII-digested lambda DNA for sizing) were found for the various species. The blots for all of the species (including both for *D. melanogaster*), except yeast, mouse and rabbit appeared to have more than one band.

For the analysis of human DNA, the placental DNA was restricted with BamHI, BglII, EcoRI, HindIII, PstI and PvuII, separated in a 0.8% agarose gel (10 µg per lane) and transferred to nitrocellulose (see McDonnell et al., supra) and hybridized as described below.

For the zooblot, EcoRI-digested DNA from the several species (Clontech, Palo Alto, California, USA), other than *D. melanogaster*, was used for Southern blot analysis. EcoRI- and XhoI-digested *D. melanogaster* DNA was included also.

Blots were hybridized at 42°C in the low stringency buffer described in Example I or at high stringency in the same buffer modified by addition of formamide to 50 %. Low stringency blots were washed twice at room temperature and twice at 50°C in 2X SSC, 0.1% SDS. The high stringency blot was washed twice at room temperature in 2X SSC, 0.1% SDS and twice at 65°C in 0.5X SSC, 0.1% SDS.

Example VII

10

Northern analysis were carried out on the mouse RXR isoforms alpha, beta and gamma, to determine the tissue distribution of these receptors in adult tissues and in developing embryos.

15

Thus, mRNA (10µg) was isolated from various adult rat tissues of from day 10.5-day 18.5 p.c. whole mouse embryos. These samples were subjected to Northern analysis using ³²P-labeled cDNA probes derived from regions specific to mRXRα, mRXRβ, or mRXRγ.

20

In the adult, the various RXR isoforms are seen to be expressed in both a specific and overlapping distribution pattern.

25

In the embryo, the various isoforms are highly expressed in what appears to be a specific temporal pattern.

30

The invention has been described in detail with particular reference to preferred embodiments thereof, but it will be understood that variations and modifications can be effected within the spirit and scope of the invention.

Deposit

On January 31, 1990, a sample of replicatable phagescript SK doubl -stranded DNA (Stratagene, La Jolla, California, USA), with the 1860 base-pair, EcoRI-site-
5 bounded DNA, the sequence of which is illustrated in

Figure 1, inserted at the unique EcoRI site, was deposited under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure at the American Type
10 Culture Collection, Rockville, Maryland, USA ("ATCC"). The accession number assigned to this deposit is ATCC 40741. The deposited DNA is designated pSK(hRXR-alpha).

Phagescript SK double-stranded DNA is a modified M13mp18 bacteriophage DNA (double-stranded). Derivatives,
15 such as pSK(hRXR-alpha), of phagescript SK double-stranded DNA can be cloned in the same way as M13mp18 and its derivatives.

Samples of pSK(hRXR-alpha) will be publicly available from the ATCC without restriction, except as provided in
20 37 CFR 1.801 et seq., at the latest on the date an United States Patent first issues on this application or a continuing application thereof. Otherwise, in accordance with the Budapest Treaty and the regulations promulgated thereunder, samples will be available from the
25 ATCC to all persons legally entitled to receive them under the law and regulations of any country or international organization in which an application, claiming priority of this application, is filed or in which a patent based on any such application is granted.

SUMMARY OF SEQUENCES

Sequence ID No. 1 is the coding sequence of an EcoRI-site-bounded DNA segment which encodes the novel receptor disclosed herein, referred to as human RXR-alpha [hRXR α]

5

Sequence ID No. 2 is the amino acid sequence of the novel receptor referred to herein as hRXR α .

Sequence ID No. 3 is the nucleotide (and amino acid) sequence of the novel receptor disclosed herein, referred to as mouse RXR-alpha [mRXR α].

10

Sequence ID No. 4 is the amino acid sequence of the novel receptor referred to herein as mRXR α .

Sequence ID No. 5 is the nucleotide (and amino acid) sequence of the novel receptor disclosed herein, referred to as mouse RXR-gamma [mRXR γ].

15

Sequence ID No. 6 is the amino acid sequence of the novel receptor referred to herein as mRXR γ .

Sequence ID No. 7 is the nucleotide sequence of the receptor disclosed by Hamada, et al in PNAS 86: 8298-8293 (1989). This receptor is similar to the receptor referred to herein as mRXR β .

20

SEQ ID NO:1:

	GAATTCGGGC GCCGGGGGCC GCCCGCCCGC CGCCCGCTGC CTGCGCGGCC GGGCGGGCAT	60
5	GAGTTAGTCG CAGAC ATG GAC ACC AAA CAT TTC CTG CCG CTC GAT TTC TCC Met Asp Thr Lys His Phe Leu Pro Leu Asp Phe Ser 1 5 10	111
10	ACC CAG GTG AAC TCC TCC CTC ACC TCC CCG ACG GGG CGA GGC TCC ATG Thr Gln Val Asn Ser Ser Leu Thr Ser Pro Thr Gly Arg Gly Ser Met 15 20 25	159
15	GCT GCC CCC TCG CTG CAC CCG TCC CTG GGG CCT GGC ATC GGC TCC CCG Ala Ala Pro Ser Leu His Pro Ser Leu Gly Pro Gly Ile Gly Ser Pro 30 35 40	207
20	GGA CAG CTG CAT TCT CCC ATC AGC ACC CTG AGC TCC CCC ATC AAC GGC Gly Gln Leu His Ser Pro Ile Ser Thr Leu Ser Ser Pro Ile Asn Gly 45 50 55 60	255
25	ATG GGC CCG CCT TTC TCG GTC ATC AGC TCC CCC ATG GGC CCC CAC TCC Met Gly Pro Pro Phe Ser Val Ile Ser Ser Pro Met Gly Pro His Ser 65 70 75	303
30	ATG TCG GTG CCC ACC ACA CCC ACC CTG GGC TTC AGC ACT GGC AGC CCC Met Ser Val Pro Thr Thr Pro Thr Leu Gly Phe Ser Thr Gly Ser Pro 80 85 90	351
35	CAG CTC AGC TCA CCT ATG AAC CCC GTC AGC AGC AGC GAG GAC ATC AAG Gln Leu Ser Ser Pro Met Asn Pro Val Ser Ser Ser Glu Asp Ile Lys 95 100 105	399
40	CCC CCC CTG GGC CTC AAT GGC GTC CTC AAG GTC CCC GCC CAC CCC TCA Pro Pro Leu Gly Leu Asn Gly Val Leu Lys Val Pro Ala His Pro Ser 110 115 120	447
45	GGA AAC ATG GCT TCC TTC ACC AAG CAC ATC TGC GCC ATC TGC GGG GAC Gly Asn Met Ala Ser Phe Thr Lys His Ile Cys Ala Ile Cys Gly Asp 125 130 135 140	495
50	CGC TCC TCA GGC AAG CAC TAT GGA GTG TAC AGC TGC GAG GGG TGC AAG Arg Ser Ser Gly Lys His Tyr Gly Val Tyr Ser Cys Glu Gly Cys Lys 145 150 155	543
55	GGC TTC TTC AAG CCG ACG GTG CGC AAG GAC CTG ACC TAC ACC TGC CGC Gly Phe Phe Lys Arg Thr Val Arg Lys Asp Leu Thr Tyr Thr Cys Arg 160 165 170	591
60	GAC AAC AAG GAC TGC CTG ATT GAC AAG CCG CAG CCG AAC CCG TGC CAG Asp Asn Lys Asp Cys Leu Ile Asp Lys Arg Gln Arg Asn Arg Cys Gln 175 180 185	639
65	TAC TGC CGC TAC CAG AAG TGC CTG GCC ATG GGC ATG AAG CCG GAA GCC Tyr Cys Arg Tyr Gln Lys Cys Leu Ala Met Gly Met Lys Arg Glu Ala 190 195 200	687
70	GTG CAG GAG GAG CCG CAG CGT GGC AAG GAC CCG AAC GAG AAT GAG GTG Val Gln Glu Glu Arg Gln Arg Gly Lys Asp Arg Asn Glu Asn Glu Val 205 210 215 220	735
75	GAG TCG ACC AGC AGC GCC AAC GAG GAC ATG CCG GTG GAG AGG ATC CTG Glu Ser Thr Ser Ser Ala Asn Glu Asp Met Pro Val Glu Arg Ile Leu 225 230 235	783
80	GAG GCT GAG CTG GCC GTG GAG CCC AAG ACC GAG ACC TAC GTG GAG GCA Glu Ala Glu Leu Ala Val Glu Pro Lys Thr Glu Thr Tyr Val Glu Ala 240 245 250	831
85	AAC ATG GGG CTG AAC CCC AGC TCG CCG AAC GAC CCT GTC ACC AAC ATT Asn Met Gly Leu Asn Pro Ser Ser Pro Asn Asp Pro Val Thr Asn Il 255 260 265	879

	TGC CAA GCA GCC GAC AAA CAG CTT TTC ACC CTG GTG GAG TGG GCC AAG Cys Gln Ala Ala Asp Lys Gln Leu Phe Thr Leu Val Glu Trp Ala Lys 270 275 280	927
5	CGG ATC CCA CAC TTC TCA GAG CTG CCC CTG GAC GAC CAG GTC ATC CTG Arg Ile Pro His Phe Ser Glu Leu Pro Leu Asp Asp Gln Val Ile Leu 285 290 295 300	975
10	CTG CGG GCA GGC TGG AAT GAG CTG CTC ATC GCC TCC TTC TCC CAC CGC Leu Arg Ala Gly Trp Asn Glu Leu Leu Ile Ala Ser Phe Ser His Arg 305 310 315	1023
15	TCC ATC GCC GTG AAG GAC GGG ATC CTC CTG GCC ACC GGG CTG CAC GTC Ser Ile Ala Val Lys Asp Gly Ile Leu Leu Ala Thr Gly Leu His Val 320 325 330	1071
	CAC CGG AAC AGC GCC CAC AGC GCA GGG GTG GGC GCC ATC TTT GAC AGG His Arg Asn Ser Ala His Ser Ala Gly Val Gly Ala Ile Phe Asp Arg 335 340 345	1119
20	GTG CTG ACG GAG CTT GTG TCC AAG ATG CGG GAC ATG CAG ATG GAC AAG Val Leu Thr Glu Leu Val Ser Lys Met Arg Asp Met Gln Met Asp Lys 350 355 360	1167
25	ACG GAG CTG GGC TGC CTG CGC GCC ATC GTC CTC TTT AAC CCT GAC TCC Thr Glu Leu Gly Cys Leu Arg Ala Ile Val Leu Phe Asn Pro Asp Ser 365 370 375 380	1215
30	AAG GGG CTC TCG AAC CCG GCC GAG GTG GAG GCG CTG AGG GAG AAG GTC Lys Gly Leu Ser Asn Pro Ala Glu Val Glu Ala Leu Arg Glu Lys Val 385 390 395	1263
35	TAT GCG TCC TTG GAG GCC TAC TGC AAG CAC AAG TAC CCA GAG CAG CCG Tyr Ala Ser Leu Glu Ala Tyr Cys Lys His Lys Tyr Pro Glu Gln Pro 400 405 410	1311
	GGA AAG TTC GCT AAG CTC TTG CTC CGC CTG CCG GCT CTG CGC TCC ATC Gly Arg Phe Ala Lys Leu Leu Arg Leu Pro Ala Leu Arg Ser Ile 415 420 425	1359
40	GGG CTC AAA TGG CTG GAA CAT CTC TTC TTC TTC AAG CTC ATC GGG GAC Gly Leu Lys Cys Leu Glu His Leu Phe Phe Phe Lys Leu Ile Gly Asp 430 435 440	1407
45	ACA CCC ATT GAC ACC TTC CTT ATG GAG ATG CTG GAG GCG CCG CAC CAA Thr Pro Ile Asp Thr Phe Leu Met Glu Met Leu Glu Ala Pro His Gln 445 450 455 460	1455
50	ATG ACT TAGGCCTGGG GGCCCATCCT TTGTGCCAC CCGTTCTGGC CACCCTGCCT Met Thr	1511
	GGAGCCGAGC TGTTCTTCTC AGCCTGAGCC CTGTCCCTGC CTTTCTCTGC CTGGCCTGTT	1571
55	TGGACTTTGG GGCACAGCCT GTCACTGCTC TGCTAAGAG ATGTGTTGTC ACCCTCCTTA TTTCTGTTAC TACTTGCTG TGGCCAGGG CAGTGGCTTT CCGAGCAGC AGCCTTCGTG	1631 1691
60	GCAAGAACTA GCGTGAGCCC AGCCAGGCC CTCCCACCG GGCTCTCAGG AGCCCTGCC ACACCCACGG GCGTTGGGG ACTACAGGT CTTCGGCCCC AGCCTGGAG CTGCAGGAGT TGGGAACGGG GCTTTTGTTC CCGTTGCTGT TTATCGATGC TGGTTTTCAG AATTC	1751 1811 1866

SEQ ID NO:2:

Met Asp Thr Lys His Ph Leu Pr Leu Asp Phe Ser Thr Gln Val Asn
 1 5 10 15
 5 Ser Ser Leu Thr Ser Pro Thr Gly Arg Gly Ser Met Ala Ala Pro Ser
 20 25 30
 10 Leu His Pro Ser Leu Gly Pro Gly Ile Gly Ser Pro Gly Gln Leu His
 35 40 45
 Ser Pro Ile Ser Thr Leu Ser Ser Pro Ile Asn Gly Met Gly Pro Pro
 50 55 60
 15 Phe Ser Val Ile Ser Ser Pro Met Gly Pro His Ser Met Ser Val Pro
 65 70 75 80
 Thr Thr Pro Thr Leu Gly Phe Ser Thr Gly Ser Pro Gln Leu Ser Ser
 85 90 95
 20 Pro Met Asn Pro Val Ser Ser Ser Glu Asp Ile Lys Pro Pro Leu Gly
 100 105 110
 Leu Asn Gly Val Leu Lys Val Pro Ala His Pro Ser Gly Asn Met Ala
 115 120 125
 Ser Phe Thr Lys His Ile Cys Ala Ile Cys Gly Asp Arg Ser Ser Gly
 130 135 140
 30 Lys His Tyr Gly Val Tyr Ser Cys Glu Gly Cys Lys Gly Phe Phe Lys
 145 150 155 160
 Arg Thr Val Arg Lys Asp Leu Thr Tyr Thr Cys Arg Asp Asn Lys Asp
 165 170 175
 35 Cys Leu Ile Asp Lys Arg Gln Arg Asn Arg Cys Gln Tyr Cys Arg Tyr
 180 185 190
 40 Gln Lys Cys Leu Ala Met Gly Met Lys Arg Glu Ala Val Gln Glu Glu
 195 200 205
 Arg Gln Arg Gly Lys Asp Arg Asn Glu Asn Glu Val Glu Ser Thr Ser
 210 215 220
 45 Ser Ala Asn Glu Asp Met Pro Val Glu Arg Ile Leu Glu Ala Glu Leu
 225 230 235 240
 Ala Val Glu Pro Lys Thr Glu Thr Tyr Val Glu Ala Asn Met Gly Leu
 245 250 255
 50 Asn Pro Ser Ser Pro Asn Asp Pro Val Thr Asn Ile Cys Gln Ala Ala
 260 265 270
 55 Asp Lys Gln Leu Phe Thr Leu Val Glu Trp Ala Lys Arg Ile Pro His
 275 280 285
 Phe Ser Glu Leu Pro Leu Asp Asp Gln Val Ile Leu Leu Arg Ala Gly
 290 295 300
 60 Trp Asn Glu Leu Leu Ile Ala Ser Phe Ser His Arg Ser Ile Ala Val
 305 310 315 320
 Lys Asp Gly Ile Leu Leu Ala Thr Gly Leu His Val His Arg Asn Ser
 325 330 335
 65 Ala His Ser Ala Gly Val Gly Ala Ile Phe Asp Arg Val Leu Thr Glu
 340 345 350
 70 Leu Val Ser Lys Met Arg Asp Met Gln Met Asp Lys Thr Glu Leu Gly
 355 360 365
 Cys Leu Arg Ala Ile Val Leu Phe Asn Pro Asp Ser Lys Gly Leu Ser
 370 375 380

28

Asn Pro Ala Glu Val Glu Ala Leu Arg Glu Lys Val Tyr Ala Ser Leu
385 390 395 400

5 Glu Ala Tyr Cys Lys His Lys Tyr Pro Glu Gln Pro Gly Arg Phe Ala
405 410 415

Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg Ser Ile Gly Leu Lys Cys
420 425 430

10 Leu Glu His Leu Phe Phe Phe Lys Leu Ile Gly Asp Thr Pro Ile Asp
435 440 445

15 Thr Phe Leu Met Glu Met Leu Glu Ala Pro His Gln Met Thr
450 455 460

SER ID NO:3:

	GAATTCGGG CCGCGGCGAC TTTTGCAACA ACTCGCGCGG CCGCGGCGTC CCGCGGCGCG	60
5	CGCGCGCGCT GCGCGCGCGG GCTCCCCGCG GCCCGGGGCC CCGCGGCGGC GCGCGGCGGG	120
	CCGCGGCGCT GCGCGCGTGC TGCTCCGCGG CCGGCTGGGC ATGAGTTAGT CCGAGAC	177
10	ATG GAC ACC AAA CAT TTC CTG CCG CTC GAC TTC TCT ACC CAG GTG AAC Met Asp Thr Lys His Phe Leu Pro Leu Asp Phe Ser Thr Gln Val Asn	225
	1 5 10 15	
15	TCT TCG TCC CTC AAC TCT CCA ACG GGT CGA GGC TCC ATG GCT GTC CCC Ser Ser Ser Leu Asn Ser Pro Thr Gly Arg Gly Ser Met Ala Val Pro	273
	20 25 30	
20	TCG CTG CAC CCC TCC TTG GGT CCG GGA ATC GGC TCT CCA CTG GGC TCG Ser Leu His Pro Ser Leu Gly Pro Gly Ile Gly Ser Pro Leu Gly Ser	321
	35 40 45	
25	CCT GGG CAG CTG CAC TCT CCT ATC AGC ACC CTG AGC TCC CCC ATC AAT Pro Gly Gln Leu His Ser Pro Ile Ser Thr Leu Ser Ser Pro Ile Asn	369
	50 55 60	
30	GGC ATG GGT CCG CCC TTC TCT GTC ATC AGC TCC CCC ATG GGC CCG CAC Gly Met Gly Pro Pro Phe Ser Val Ile Ser Ser Pro Met Gly Pro His	417
	65 70 75 80	
35	TCC ATG TCG GTA CCC ACC ACA CCC ACA TTG GGC TTC GGG ACT GGT AGC Ser Met Ser Val Pro Thr Thr Pro Thr Leu Gly Phe Gly Thr Gly Ser	465
	85 90 95	
40	CCC CAG CTC AAT TCA CCC ATG AAC CCT GTG AGC AGC ACT GAG GAT ATC Pro Gln Leu Asn Ser Pro Met Asn Pro Val Ser Ser Thr Glu Asp Ile	513
	100 105 110	
45	AAG CCG CCA CTA GGC CTC AAT GGC GTC CTC AAG GTT CCT GCC GAT CCC Lys Pro Pro Leu Gly Leu Asn Gly Val Leu Lys Val Pro Ala His Pro	561
	115 120 125	
50	TCA GGA AAT ATG GCC TCC TTC ACC AAG CAC ATC TGT GCT ATC TGT GGG Ser Gly Asn Met Ala Ser Phe Thr Lys His Ile Cys Ala Ile Cys Gly	609
	130 135 140	
55	GAC CGC TCC TCA GGC AAA CAC TAT GGG GTA TAC AGT TGT GAG GGC TGC Asp Arg Ser Ser Gly Lys His Tyr Gly Val Tyr Ser Cys Glu Gly Cys	657
	145 150 155 160	
60	AAG GGC TTC TTC AAG AGG ACA GTA CCG AAA GAC CTG ACC TAC ACC TGC Lys Gly Phe Phe Lys Arg Thr Val Arg Lys Asp Leu Thr Tyr Thr Cys	705
	165 170 175	
65	CGA GAC AAC AAG GAC TGC CTG ATG GAC AAG AGA CAG CGG AAC CCG TGT Arg Asp Asn Lys Asp Cys Leu Ile Asp Lys Arg Gln Arg Asn Arg Cys	753
	180 185 190	
70	CAG TAC TGC CGC TAC CAG AAG TGC CTG GCC ATG GGC ATG AAG CCG GAA Gln Tyr Cys Arg Tyr Gln Lys Cys Leu Ala Met Gly Met Lys Arg Glu	801
	195 200 205	
75	GCT GTG CAG GAG GAG CCG CAG CCG GGC AAG GAC CCG AAT GAG AAC GAG Ala Val Gln Glu Glu Arg Gln Arg Gly Lys Asp Arg Asn Glu Asn Glu	849
	210 215 220	
80	GTG GAG TCC ACC AGC AGT GCC AAC GAG GAC ATG CCT GTA GAG AAG ATT Val Glu Ser Thr Ser Ser Ala Asn Glu Asp Met Pro Val Glu Lys Ile	897
	225 230 235 240	
85	CTG GAA GCC GAG CTT GCT GTC GAG CCC AAG ACT GAG ACA TAC GTG GAG Leu Glu Ala Glu Ala Val Glu Pro Lys Thr Glu Thr Tyr Val Glu	945
	245 250 255	

	GCA AAC ATG GGG CTG AAC CCC AGC TCA CCA AAT GAC CCT GTT ACC AAC Ala Asn Met Gly Leu Asn Pro Ser Ser Pro Asn Asp Pro Val Thr Asn 260 265 270	993
5	ATC TGT CAA GCA GCA GAC AAG CAG CTC TTC ACT CTT GTG GAG TGG GCC Ile Cys Gln Ala Ala Asp Lys Gln Leu Phe Thr Leu Val Glu Trp Ala 275 280 285	1041
10	AAG AGG ATC CCA CAC TTT TCT GAG CTG CCC CTA GAC GAC CAG GTC ATC Lys Arg Ile Pro His Phe Ser Glu Leu Pro Leu Asp Asp Gln Val Ile 290 295 300	1089
15	CTG CTA CGG GCA GGC TGG AAC GAG CTG CTG ATC GCC TCC TTC TCC CAC Leu Leu Arg Ala Gly Trp Asn Glu Leu Leu Ile Ala Ser Phe Ser His 305 310 315 320	1137
20	CGC TCC ATA GCT GTG AAA GAT GGG ATT CTC CTG GCC ACC GGG CTG CAC Arg Ser Ile Ala Val Lys Asp Gly Ile Leu Leu Ala Thr Gly Leu His 325 330 335	1185
25	GTA CAC CGG AAC AGC GCT CAC AGT GCT GGG GTG GGC GCC ATC TTT GAC Val His Arg Asn Ser Ala His Ser Ala Gly Val Gly Ala Ile Phe Asp 340 345 350	1233
30	AGG GTG CTA ACA GAG CTG GTG TCT AAG ATG CGT GAC ATG CAG ATG GAC Arg Val Leu Thr Glu Leu Val Ser Lys Met Arg Asp Met Gln Met Asp 355 360 365	1281
35	AAG ACG GAG CTG GGC TGC CTG CGA GCC ATT GTC CTG TTC AAC CCT GAC Lys Thr Glu Leu Gly Cys Leu Arg Ala Ile Val Leu Phe Asn Pro Asp 370 375 380	1329
40	TCT AAG GGG CTC TCA AAC CCT GCT GAG GTG GAG GCG TTG AGG GAG AAG Ser Lys Gly Leu Ser Asn Pro Ala Glu Val Glu Ala Leu Arg Glu Lys 385 390 395 400	1377
45	GTG TAT GCG TCA CTA GAA GCG TAC TGC AAA CAC AAG TAC CCT GAG CAG Val Tyr Ala Ser Leu Glu Ala Tyr Cys Lys His Lys Tyr Pro Glu Gln 405 410 415	1425
50	CCG GGC AGG TTT GCC AAG CTG CTG CTC CGC CTG CCT GCA CTG CGT TCC Pro Gly Arg Phe Ala Lys Leu Leu Arg Leu Pro Ala Leu Arg Ser 420 425 430	1473
55	ATC GGG CTC AAG TGC CTG GAG CAC CTG TTC TTC TTC AAG CTC ATC GGG Ile Gly Leu Lys Cys Leu Glu His Leu Phe Phe Phe Lys Leu Ile Gly 435 440 445	1521
60	GAC ACG CCC ATC GAC ACC TTC CTC ATG GAG ATG CTG GAG GCA CCA CAT Asp Thr Pro Ile Asp Thr Phe Leu Met Glu Met Leu Glu Ala Pro His 450 455 460	1569
65	CAA GGC ACC TAGGCCCCCG CCGCCGTGTG CCGGTCCCGT GCCCTGCCTG Gln Ala Thr 465	1618
70	GACACAGCTG CTCAGCTCCA GCCCTGCCCC TGCCTTTTCT GATGGCCCGT GTGGATCTTT GGGGTGCACT GTCCCTATGG GCCCAAAGA TGCATCACCA TCCTCGCCAT CTTTACTCAT GCTTGCCCTT GCCCAGGGC ATAGCAGAGC TGCTGTGACA CCTGGCCAGC TCCTGCCCTA CATCAGGCTC TAAGGTATG CTGCTGTAC CCGAGGGTC GTGGGGTTCC TCATGGGGCC TTCAGCACT GGAGTGCAA GAGCTGGGA AAGGCTTGT TGTGTTTGT GTTGCTGCT CGCTGGTTCT CGACATCCA CATGGCACT CTGTTTGGAG TGCCCATCT TGGCTGTTC AGAGTCCTGG TACCCAGTTA GGGTGGGAAT CCACCTGGGA TCAAGAAGGA GCAGGTGGGG CAGGCCGTAT CCTCCTGGGT CATAGCTAAC CTATAAAGGC GCCCGAATT CCTCGAG	1678 1738 1798 1858 1918 1978 2038 2095

SEQ ID NO:4:

Met Asp Thr Lys His Phe Leu Pro Leu Asp Phe Ser Thr Gln Val Asn
 1 5 10 15
 Ser Ser Ser Leu Asn Ser Pro Thr Gly Arg Gly Ser Met Ala Val Pro
 20 25 30
 Ser Leu His Pro Ser Leu Gly Pro Gly Ile Gly Ser Pro Leu Gly Ser
 35 40 45
 Pro Gly Gln Leu His Ser Pro Ile Ser Thr Leu Ser Ser Pro Ile Asn
 50 55 60
 Gly Met Gly Pro Pro Phe Ser Val Ile Ser Ser Pro Met Gly Pro His
 65 70 75 80
 Ser Met Ser Val Pro Thr Thr Pro Thr Leu Gly Phe Gly Thr Gly Ser
 85 90 95
 Pro Gln Leu Asn Ser Pro Met Asn Pro Val Ser Ser Thr Glu Asp Ile
 100 105 110
 Lys Pro Pro Leu Gly Leu Asn Gly Val Leu Lys Val Pro Ala His Pro
 115 120 125
 Ser Gly Asn Met Ala Ser Phe Thr Lys His Ile Cys Ala Ile Cys Gly
 130 135 140
 Asp Arg Ser Ser Gly Lys His Tyr Gly Val Tyr Ser Cys Glu Gly Cys
 145 150 155 160
 Lys Gly Phe Phe Lys Arg Thr Val Arg Lys Asp Leu Thr Tyr Thr Cys
 165 170 175
 Arg Asp Asn Lys Asp Cys Leu Ile Asp Lys Arg Gln Arg Asn Arg Cys
 180 185 190
 Gln Tyr Cys Arg Tyr Gln Lys Cys Leu Ala Met Gly Met Lys Arg Glu
 195 200 205
 Ala Val Gln Glu Glu Arg Gln Arg Gly Lys Asp Arg Asn Glu Asn Glu
 210 215 220
 Val Glu Ser Thr Ser Ser Ala Asn Glu Asp Met Pro Val Glu Lys Ile
 225 230 235 240
 Leu Glu Ala Glu Leu Ala Val Glu Pro Lys Thr Glu Thr Tyr Val Glu
 245 250 255
 Ala Asn Met Gly Leu Asn Pro Ser Ser Pro Asn Asp Pro Val Thr Asn
 260 265 270
 Ile Cys Gln Ala Ala Asp Lys Gln Leu Phe Thr Leu Val Glu Trp Ala
 275 280 285
 Lys Arg Ile Pro His Phe Ser Glu Leu Pro Leu Asp Asp Gln Val Ile
 290 295 300
 Leu Leu Arg Ala Gly Trp Asn Glu Leu Leu Ile Ala Ser Phe Ser His
 305 310 315 320
 Arg Ser Ile Ala Val Lys Asp Gly Ile Leu Leu Ala Thr Gly Leu His
 325 330 335
 Val His Arg Asn Ser Ala His Ser Ala Gly Val Gly Ala Ile Phe Asp
 340 345 350
 Arg Val Leu Thr Glu Leu Val Ser Lys Met Arg Asp Met Gln Met Asp
 355 360 365
 Lys Thr Glu Leu Gly Cys Leu Arg Ala Il Val Leu Phe Asn Pro Asp
 370 375 380

	Ser Lys Gly Leu Ser Asn Pro Ala Glu Val Glu Ala Leu Arg Glu Lys	
	385	400
		390
5	Val Tyr Ala Ser Leu Glu Ala Tyr Cys Lys His Lys Tyr Pro Glu Gln	
	405	415
		410
	Pro Gly Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg Ser	
	420	430
		425
10	Ile Gly Leu Lys Cys Leu Glu His Leu Phe Phe Phe Lys Leu Ile Gly	
	435	445
		440
	Asp Thr Pro Ile Asp Thr Phe Leu Met Glu Met Leu Glu Ala Pro His	
	450	460
		455
15	Gln Ala Thr	
	465	

SER ID NO:5:

	GAATTCGGGG CCGCGCTGTG CCTGGGAGCC GAGAGAGAGA GAGAGAGAGA GAGAGAGAGA	60
5	GAGAGAGAGA GAGAGGCTGT ACTCTTCAGA AGCGCACGAG AGGAATGAAC TGAGCAGCCA	120
	AC ATG TAT GGA AAT TAT TCC CAC TTC ATG AAG TTT CCC ACC GGC TTT Met Tyr Gly Asn Tyr Ser His Phe Met Lys Phe Pro Thr Gly Phe	167
10	GGT GGC TCC CCT GGT CAC ACT GGC TCG ACC TCC ATG AGC CCT TCA GTA Gly Gly Ser Pro Gly His Thr Gly Ser Thr Ser Met Ser Pro Ser Val	215
15	GCC TTG CCC ACC GGG AAG CCA ATG GAC AGC GAC CCC AGC TAC ACA GAC Ala Leu Pro Thr Gly Lys Pro Met Asp Ser His Pro Ser Tyr Thr Asp	263
20	ACC CCA GTG AGT GCC CCT CGG ACC CTG AGT GCT GTG GGA ACC CCC CTC Thr Pro Val Ser Ala Pro Arg Thr Leu Ser Ala Val Gly Thr Pro Leu	311
25	AAT GCT CTT GGC TCT CCG TAT AGA GTC ATC ACT TCT GCC ATG GGT CCA Asn Ala Leu Gly Ser Pro Tyr Arg Val Ile Thr Ser Ala Met Gly Pro	359
30	CCC TCA GGA GCA CTG GCA GCT CCT CCA GGA ATC AAC TTG GTG GCT CCA Pro Ser Gly Ala Leu Ala Pro Pro Gly Ile Asn Leu Val Ala Pro	407
35	CCC AGC TCC CAG CTA AAT GTG GTC AAC AGT GTC AGC AGC TCT GAG GAC Pro Ser Ser Gln Leu Asn Val Val Asn Ser Val Ser Ser Ser Glu Asp	455
40	ATC AAG CCC TTA CCA GGT CTG CCT GGG ATT GGA AAT ATG AAC TAC CCA Ile Lys Pro Gly Leu Pro Gly Ile Gly Asn Met Asn Tyr Pro	503
45	TCC ACC AGC CCT GGG TCT CTG GTG AAA CAC ATC TGT GCC ATC TGT GGG Ser Thr Ser Pro Gly Ser Leu Val Lys His Ile Cys Ala Ile Cys Gly	551
50	GAC AGA TCC TCA GGG AAG CAC TAC GGT GTG TAC AGC TGT GAA GGT TGC Asp Arg Ser Ser Gly Lys His Tyr Gly Val Tyr Ser Cys Glu Gly Cys	599
55	AAA GGC TTC TTC AAA AGG ACC ATC AGG AAA GAT CTC ATC TAC ACC TGT Lys Gly Phe Phe Lys Arg Thr Ile Arg Lys Asp Leu Ile Tyr Thr Cys	647
60	CGG GAT AAC AAA GAT TGT CTC ATC GAC AAG CGC CAG CGC AAC CGC TGC Arg Asp Asn Lys Asp Cys Leu Ile Asp Lys Arg Gln Arg Asn Arg Cys	695
65	CAG TAC TGT CGC TAC CAG AAG TGC CTG GTC ATG GGC ATG AAG CGG GAA Gln Tyr Cys Arg Tyr Gln Lys Cys Leu Val Met Gly Met Lys Arg Glu	743
70	GCT GTG CAA GAA GAA AGG CAG AGG AGC CGA GAG CGA GCA GAG AGT GAG Ala Val Gln Glu Glu Arg Gln Arg Ser Arg Glu Arg Ala Glu Ser Glu	791
	GCA GAA TGT GGC AGT AGT AGC CAC GAA GAC ATG CCC GTG GAG AGC ATT Ala Glu Cys Ala Ser Ser Ser His Glu Asp Met Pro Val Glu Arg Ile	839
	CTA GAA GCC GAA CTT GCT GTG GAA CCA AAG ACA GAA TCC TAC GGT GAC Leu Glu Ala Glu Leu Ala Val Glu Pro Lys Thr Glu Ser Tyr Gly Asp	887
	ATG AAC GTG GAG AAC TCA ACA AAT GAC CCT GTT ACC AAC ATA TGC CAT Met Asn Val Glu Asn Ser Thr Asn Asp Pro Val Thr Asn Ile Cys His	935

	GCT GCA GAT AAG CAA CTT TTC ACC CTC GTT GAG TGG GCC AAA CGC ATC Ala Ala Asp Lys Gln Leu Phe Thr Leu Val Glu Trp Ala Lys Arg Il 275 280 285	983
5	CCC CAC TTC TCA GAT CTC ACC TTG GAG GAC CAG GTC ATT CTA CTC CGG Pro His Phe Ser Asp Leu Thr Leu Glu Asp Gln Val Ile Leu Leu Arg 290 295 300	1031
10	GCA GGG TGG AAT GAA CTG CTC ATT GCC TCC TTC TCC CAC CGC TCG GTT Ala Gly Trp Asn Glu Leu Ile Ala Ser Phe Ser His Arg Ser Val 305 310 315	1079
15	TCC GTC CAG GAT GGC ATC CTG CTG GCC ACG GGC CTC CAC GTG CAC AGG Ser Val Gln Asp Gly Ile Leu Leu Ala Thr Gly Leu His Val His Arg 320 325 330 335	1127
20	AGC AGC GCT CAC AGC CGG GGA GTC GGC TCC ATC TTC GAC AGA GTC CTT Ser Ser Ala His Ser Arg Gly Val Gly Ser Ile Phe Asp Arg Val Leu 340 345 350	1175
	ACA GAG TTG GTG TCC AAG ATG AAA GAC ATG CAG ATG GAT AAG TCA GAG Thr Glu Leu Val Ser Lys Met Lys Asp Met Gln Met Asp Lys Ser Glu 355 360 365	1223
25	CTG GGG TGC CTA CGG GCC ATC GTG CTG TTT AAC CCA GAT GCC AAG GGT Leu Gly Cys Leu Arg Ala Ile Val Leu Phe Asn Pro Asp Ala Lys Gly 370 375 380	1271
30	TTA TCC AAC CCC TCT GAG GTG GAG ACT CTT CGA GAG AAG GTT TAT GCC Leu Ser Asn Pro Ser Glu Val Glu Thr Leu Arg Glu Lys Val Tyr Ala 385 390 395	1319
35	ACC CTG GAG GCC TAT ACC AAG CAG AAG TAT CCG GAA CAG CCA GGC AAG Thr Leu Glu Ala Tyr Thr Lys Gln Lys Tyr Pro Glu Gln Pro Gly Arg 400 405 410 415	1367
40	TTT GCC AAG CTT CTG CTG CGT CTC CCT GCT CTG CGC TCC ATC GGC TTG Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg Ser Ile Gly Leu 420 425 430	1415
	AAA TGC CTG GAA CAC CTC TTC TTC TTC AAG CTC ATT GGA GAC ACT CCC Lys Cys Leu Glu His Leu Phe Phe Phe Lys Leu Ile Gly Asp Thr Pro 435 440 445	1463
45	ATC GAC AGC TTC CTC ATG GAG ATG TTG GAG ACC CCA CTG CAG ATC ACC Ile Asp Ser Phe Leu Met Glu Met Leu Glu Thr Pro Leu Gln Ile Thr 450 455 460	1511
50	TGAACCTCCT CAGCTGCAGC TTCCCCACCC AGGCTGACCC TTGGGCGGGT GTGTGTGTGT GGCCCTACCC TGCACACTCT CCCCCATCTT CCACTCTGGC CTCCTTTCCT GTCCCCAAAA TGTGATGCTT GTAATAAGCG GCCCGAATT C	1571 1631 1662

SEQ ID NO:6:

Met Tyr Gly Asn Tyr Ser His Phe Met Lys Phe Pro Thr Gly Phe Gly
 1 5 10 15
 5 Gly Ser Pro Gly His Thr Gly Ser Thr Ser Met Ser Pro Ser Val Ala
 20 25 30
 10 Leu Pro Thr Gly Lys Pro Met Asp Ser His Pro Ser Tyr Thr Asp Thr
 35 40 45
 Pro Val Ser Ala Pro Arg Thr Leu Ser Ala Val Gly Thr Pro Leu Asn
 50 55 60
 15 Ala Leu Gly Ser Pro Tyr Arg Val Ile Thr Ser Ala Met Gly Pro Pro
 65 70 75 80
 Ser Gly Ala Leu Ala Ala Pro Pro Gly Ile Asn Leu Val Ala Pro Pro
 85 90 95
 20 Ser Ser Gln Leu Asn Val Val Asn Ser Val Ser Ser Ser Glu Asp Ile
 100 105 110
 Lys Pro Leu Pro Gly Leu Pro Gly Ile Gly Asn Met Asn Tyr Pro Ser
 115 120 125
 25 Thr Ser Pro Gly Ser Leu Val Lys His Ile Cys Ala Ile Cys Gly Asp
 130 135 140
 30 Arg Ser Ser Gly Lys His Tyr Gly Val Tyr Ser Cys Glu Gly Cys Lys
 145 150 155 160
 Gly Phe Phe Lys Arg Thr Ile Arg Lys Asp Leu Ile Tyr Thr Cys Arg
 165 170 175
 35 Asp Asn Lys Asp Cys Leu Ile Asp Lys Arg Gln Arg Asn Arg Cys Gln
 180 185 190
 40 Tyr Cys Arg Tyr Gln Lys Cys Leu Val Met Gly Met Lys Arg Glu Ala
 195 200 205
 Val Gln Glu Glu Arg Gln Arg Ser Arg Glu Arg Ala Glu Ser Glu Ala
 210 215 220
 45 Glu Cys Ala Ser Ser Ser His Glu Asp Met Pro Val Glu Arg Ile Leu
 225 230 235 240
 Glu Ala Glu Leu Ala Val Glu Pro Lys Thr Glu Ser Tyr Gly Asp Met
 245 250 255
 50 Asn Val Glu Asn Ser Thr Asn Asp Pro Val Thr Asn Ile Cys His Ala
 260 265 270
 55 Ala Asp Lys Gln Leu Phe Thr Leu Val Glu Trp Ala Lys Arg Ile Pro
 275 280 285
 His Phe Ser Asp Leu Thr Leu Glu Asp Gln Val Ile Leu Leu Arg Ala
 290 295 300
 60 Gly Trp Asn Glu Leu Leu Ile Ala Ser Phe Ser His Arg Ser Val Ser
 305 310 315 320
 Val Gln Asp Gly Ile Leu Leu Ala Thr Gly Leu His Val His Arg Ser
 325 330 335
 65 Ser Ala His Ser Arg Gly Val Gly Ser Ile Phe Asp Arg Val Leu Thr
 340 345 350
 Glu Leu Val Ser Lys Met Lys Asp Met Gln Met Asp Lys Ser Glu Leu
 355 360 365
 70 Gly Cys Leu Arg Ala Ile Val Leu Phe Asn Pro Asp Ala Lys Gly Leu
 370 375 380

Ser Asn Pro Ser Glu Val Glu Thr Leu Arg Glu Lys Val Tyr Ala Thr
385 390 395 400

5 Leu Glu Ala Tyr Thr Lys Gln Lys Tyr Pro Glu Gln Pro Gly Arg Phe
405 410 415

Ala Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg Ser Ile Gly Leu Lys
420 425 430

10 Cys Leu Glu His Leu Phe Phe Phe Lys Leu Ile Gly Asp Thr Pro Ile
435 440 445

Asp Ser Phe Leu Met Glu Met Leu Glu Thr Pro Leu Gln Ile Thr
450 455 460

15

SEQ ID NO:7:

	GAATTCCTCC GAAGCCGAGA CAGCTCTCTC CCAAATCTCC TTCTCAGGG GATCCGTCGG	60
5	TTTCTCTCTC CTGGCCGACC TCTTACCCCT TCAGCAGCTC CAGCTCCA ATG CCA CCC Met Pro Pro 1	117
10	CCG CCA CTG GGC TCC CCC TTC CCA GTC ATC AGT TCT TCC ATG GGG TCC Pro Pro Leu Gly Ser Pro Phe Pro Val Ile Ser Ser Ser Met Gly Ser 5 10 15	165
15	CCT GGT CTG CCC CCT CCG GCT CCC CCA GGA TTC TCC GGG CCT GTC AGC Pro Gly Leu Pro Pro Pro Ala Pro Pro Gly Phe Ser Gly Pro Val Ser 20 25 30 35	213
20	AGC CCT CAG ATC AAC TCC ACA GTG TCG CTC CCT GGG GGT GGG TCT GGC Ser Pro Gln Ile Asn Ser Thr Val Ser Leu Pro Gly Gly Gly Ser Gly 40 48 50	261
25	CCC CCT GAA GAT GTG AAG CCA CCG CTC TTA GGG GTC CCG GGC CTG CAC Pro Pro Glu Asp Val Lys Pro Pro Val Leu Gly Val Arg Gly Leu His 55 60 65	309
30	TGT CCA CCC CCT CCA GGT GGT CCT GGG GCT GGC AAA CCG CTC TGT GCA Cys Pro Pro Pro Pro Gly Gly Pro Gly Ala Gly Lys Arg Leu Cys Ala 70 75 80	357
35	ATC TGC GGG GAC CGA AGC TCA GGC AAG CAC TAT GGG GTT TAC AGC TGC Ile Cys Gly Asp Arg Ser Ser Gly Lys His Tyr Gly Val Tyr Ser Cys 85 90 95	405
40	GAG GGC TGC AAG GGT TTC TTC AAG CGC ACC ATT CCG AAG GAC CTG ACC Glu Gly Cys Lys Gly Phe Phe Lys Arg Thr Ile Arg Lys Asp Leu Thr 100 105 110 115	453
45	TAC TCG TGT CGT GAT AAC AAA GAC TGT ACA GTG GAC AAG CCG CAG CCG Tyr Ser Cys Arg Asp Asn Lys Asp Cys Thr Val Asp Lys Arg Gln Arg 120 125 130	501
50	AAT CGC TGT CAG TAC TGT CGC TAT CAG AAG TGC CTG GCC ACT GGC ATG Asn Arg Cys Gln Tyr Cys Arg Tyr Gln Lys Cys Leu Ala Thr Gly Met 135 140 145	549
55	AAA AGG CAG GCG GTT CAG GAG GAG TGT CAA CCG GGG AAG GAC AAA GAC Lys Arg Glu Ala Val Gln Glu Glu Arg Gln Arg Gly Lys Asp Lys Asp 150 155 160	597
60	GGG GAT GGA GAT GGG GCT GGG GGA GCC CCT GAG GAG ATG CCT GTG GAC Gly Asp Gly Asp Gly Ala Gly Gly Ala Pro Glu Glu Met Pro Val Asp 165 170 175	645
65	AGG ATC CTG GAG GCA GAG CTT GCT GTG TAG CAG AAG AGT GAG CAA GGC Arg Ile Leu Glu Ala Glu Leu Ala Val Glu Gln Lys Ser Asp Gln Gly 180 185 190 195	693
70	GTT GAG GGT CCT GGG GCC ACC GGG GGT GGT GGC AGC AGC CCA AAT GAC Val Glu Gly Pro Gly Ala Thr Gly Gly Gly Gly Ser Ser Pro Asn Asp 200 205 210	741
75	CCA GTG ACT AAC ATC TGC CAG GCA GCT GAC AAA CAG CTG TTC ACA CTC Pro Val Thr Asn Ile Cys Gln Ala Ala Asp Lys Gln Leu Phe Thr Leu 215 220 225	789
80	GTT GAG TGG GCA AAG AGG ATC CCG CAC TTC TCC TCC CTA CCT CTG GAC Val Glu Trp Ala Lys Arg Ile Pro His Phe Ser Ser Leu Pro Leu Asp 230 235 240	837
85	GAT CAG GTC ATA CTG CTG CCG GCA GGC TGG AAC GAG CTC CTC ATT GCG Asp Gln Val Ile Leu Leu Arg Ala Gly Trp Asn Glu Leu Leu Ile Ala 245 250 255	885

	TCC TTC TCC CAT CCG TCC ATT GAT GTC CGA GAT GGC ATC CTC CTG GCC Ser Phe Ser Ser His Arg Ser Ile Asp Val Arg Asp Gly Ile Leu Leu Ala 260 265 270 275	933
5	ACG GGT CTT CAT GTG CAC AGA AAC TCA GCC CAT TCC GCA GGC GTG GGA Thr Gly Leu His Val His Arg Asn Ser Ala His Ser Ala Gly Val Gly 280 285 290	981
10	GCC ATC TTT GAT CCG GTG CTG ACA GAG CTA GTG TCC AAA ATG CGT GAC Ala Ile Phe Asp Arg Val Leu Thr Glu Leu Val Ser Lys Met Arg Asp 295 300 305	1029
15	ATG AGG ATG GAC AAG ACA GAG CTT GGC TGC CTG CCG GCA ATC ATA CTG Met Arg Met Asp Lys Thr Glu Leu Gly Cys Leu Arg Ala Ile Ile Leu 310 315 320	1077
20	TTT AAT CCA GAC GCC AAG GGC CTC TCC AAC CCT GGA GAG GTG GAG ATC Phe Asn Pro Asp Ala Lys Gly Leu Ser Asn Pro Gly Glu Val Glu Ile 325 330 335	1125
25	CTT CCG GAG AAG GTG TAC GCC TCA CTG GAG ACC TAT TGC AAG CAG AAG Leu Arg Glu Lys Val Tyr Ala Ser Leu Glu Thr Tyr Cys Lys Gln Lys 340 345 350 355	1173
30	TAC CCT GAG CAG CAG GGC CCG TTT GCC AAG CTG CTG TTA CGT CTT CCT Tyr Pro Glu Gln Gly Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro 360 365 370	1221
35	GCC CTC CCG TCC ATC GGC CTC AAG TGT CTG GAG CAC CTG TTC TTC TTC Ala Leu Arg Ser Ile Gly Leu Lys Cys Leu Glu His Leu Phe Phe Phe 375 380 385	1269
40	AAG CTC ATT GGC GAC ACC CCC ATT GAC ACC TTC CTC ATG GAG ATG CTT Lys Leu Ile Gly Asp Thr Pro Ile Asp Thr Phe Leu Met Glu Met Leu 390 395 400	1317
45	GAG GCT CCC CAC CAG CTA GCC TGAGCCCAGA TGCACACCGA GTGTCACTGA Glu Ala Pro His Gln Leu Ala 405 410	1368
50	GGAGGACTTG AGCCTGGGCA GGGGGCAGAG CCATGGGACA GGTGCAGAGC AGGAGGGGAC TTGCCAGGCC TGCCAGGGAT CTGGCAACAC TTAGCAGGGT TCCTTGGTC TCCAAGTCCA	1428 1488
55	AGGGGACCCC AGATCCCTGT GAGGACTTTA TGTCTACCTT CAGTGGCCTT GAGTCTCTGA ATTTGTGGGG GTCTCCCATG GTGCAGGTGA TTCTTCATCC TGGCTCCCCA GCACAAAGCA	1548 1608
60	CTGCCCTGCT TCCTTCTCAT TTGGCCTCAC TCCCTTCTGA AGAGTGGAAC AGAGCTCCCC CAGAAAGGGG TGTGTGGGG CAGGCCCCC AAGCTGATGA TCATGGGAGC AGGCTCTGA	1668 1728
65	CAGCCTTTAT CCTCTCAGAC TTGACAGATG GGGGCAGAGG AGGGACCTGC CTCTGTCTCC TGTCAGCCCC ATTTCCACAG TCCCTCCTGC AGTCAGACTG AAGAATAAAG GGGTAGTGAA	1788 1848
70	GGGGCTGCTG GAGGTGGAGG AACCCATTGC TCTTTTAATT TCCTGTGAGG AGAGACTGGG AGTTAGACTC AAAGAAGTAC TGTACATCCC CAGGTTGACT TAAATGTCAG GGCTGGAGAT GGCATGTGGG CAAGGAGGCC CCTCAGGTGG GCTGTCCCAA AGCTCCCTGG GCTCTGCCCTC GGGTGGCCCT ACAGCTCTTC CTAGTCTTA AGCACAGCTA GGCTGGGAGC AAGTGGGGAC ATTGATGGGG GTGGCCAGCC TGCAGAGTTG GGTGCTGGGC TGCATGGTTT TTGCCCTGGA CCTCTTTTGG GGGTCCCTC CCATCTTTCA CTGCACTA AAGTTGCTTT CCAGTTAAAA AAAAAAAA A	1908 1968 2028 2088 2148 2208 2219

CLAIMS

That which is claim d is:

1. A substantially pure DNA sequence which encodes
5 a polypeptide, wherein said polypeptide is characterized
by:

- (1) being responsive to the presence of retinoid(s)
to regulate the transcription of associated
gene(s);
- 10 (2) having a DNA binding domain of about 66 amino
acids with 9 Cys residues, wherein said DNA
binding domain has:
 - (a) less than about 65 % amino acid identity
with the DNA binding domain of hRAR-alpha,
 - 15 (b) less than about 55 % amino acid identity
with the DNA binding domain of hTR-beta,
and
 - (c) less than about 55 % amino acid identity
20 with the DNA binding domain of hGR; and
- (3) not including the sequence set forth in
Sequence ID No 7.

2. A DNA sequence according to Claim 1 wherein said
25 polypeptide is encoded by a continuous sequence which
encodes substantially the same sequence as that of:

- amino acids 1 - 462 shown in Sequence ID No. 2
[hRXR- α],
- amino acids 1 - 467 shown in Sequence ID No. 4
30 [mRXR- α], or
- amino acids 1 - 463 shown in Sequence ID No. 6
[mRXR- γ].

3. A DNA sequenc according to Claim 1 wherein said
35 polypeptide is enc d d by a continuous sequence which
encodes substantially th same sequence as that of:

amino acids 135 - 200 shown in Sequence ID No. 2
[hRXR- α],

amino acids 140 - 205 shown in Sequence ID No. 4
[mRXR- α], or

5 amino acids 139 - 204 shown in Sequence ID No. 6
[mRXR- γ].

4. A DNA sequence according to Claim 1 which
comprises a segment having a continuous nucleotide
10 sequence which is substantially the same as:

nucleotides 76 - 1464 shown in Sequence ID No. 1
[hRXR- α],

nucleotides 181 - 1581 shown in Sequence ID No. 2
[mRXR- α], or

15 nucleotides 123 - 1514 shown in Sequence ID No. 3
[mRXR- γ].

5. A DNA sequence according to Claim 4 which is
pSK(hRXR- α), pSK(mRXR- α), or pSK(mRXR- γ).

20

6. A substantially pure DNA construct comprising:

(i) the DNA sequence of Claim 1 operatively linked
to

25 (ii) regulatory element(s) operative for
transcription of said DNA sequence and
expression of said polypeptide in an animal
cell in culture.

7. A DNA construct according to Claim 6 which is
30 selected from A5C-hRXR- α , A5C-mRXR- α ,
A5C-mRXR- γ , RS-hRXR- α , RS-mRXR- α , or
RS-mRXR- γ .

8. An animal cell in culture which is transformed
35 with a DNA construct according to Claim 6.

9. A cell according to Claim 8 wherein said cell is an insect cell or a mammalian cell.

10. A cell according to Claim 9 wherein the DNA
5 construct is selected from A5C-hRXR-alpha, A5C-mRXR-alpha, A5C-mRXR-gamma, RS-hRXR-alpha, RS-mRXR-alpha, or RS-mRXR-gamma.

11. A cell according to Claim 8, wherein said cell
10 is further transformed with a reporter vector which comprises:

- (a) a promoter that is operable in said cell,
- (b) a hormone response element, and
- (c) a DNA segment encoding a reporter protein,
15 wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and wherein said hormone response element is operatively linked to said promoter for
20 activation thereof.

12. A cell according to Claim 11 wherein:
the promoter is the 5'-LTR promoter of a mouse mammary tumor virus,
25 the hormone response element is selected from TRE_p or beta-RARE, and
the reporter protein is selected from chloramphenicol acetyltransferase, luciferase, or beta-galactosidase.

13. A cell according to Claim 12 wherein the reporter vector is selected from delta-MTV-TRE_p-CAT, delta-TK-TRE_p-CAT, delta-SV-TRE_p-CAT, delta-MTV-TRE_p-LUC, delta-TK-TRE_p-LUC, or delta-SV-TRE_p-LUC.
30
35

14. A cell according to Claim 12 where in the reporter vector is selected from ADH-TRE_p-CAT, ADH-TRE_p-LUC, TK-TRE_p-CAT, or TK-TRE_p-LUC.

5 15. A cell according to Claim 14 which is a *Drosophila melanogaster* Schneider line 2 cell.

10 16. A method of testing a compound for its ability to regulate transcription-activating effects of a receptor polypeptide, said method comprising assaying for the presence or absence of reporter protein upon contacting of cells containing a receptor polypeptide and reporter vector with said compound;

15 wherein said receptor polypeptide is characterized by:

- (1) being responsive to the presence of retinoid(s) to regulate the transcription of associated gene(s); and
- 20 (2) having a DNA binding domain of about 66 amino acids with 9 Cys residues, wherein said DNA binding domain has:
 - (a) less than about 65 % amino acid identity with the DNA binding domain of hRAR-alpha,
 - 25 (b) less than about 55 % amino acid identity with the DNA binding domain of hTR-beta, and
 - (c) less than about 55 % amino acid identity with the DNA binding domain of hGR, and

30 wherein said reporter vector comprises:

- (a) a promoter that is operable in said cell,
- (b) a hormone response element, and
- (c) a DNA segment encoding a reporter protein,

wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and

wherein said hormone response element is operatively linked to said promoter for activation thereof.

17. A method according to Claim 16 wherein said contacting is carried out in the further presence of at least one retinoid species.

18. A method according to Claim 16 wherein the cells employed are CV-1 cells co-transformed with a vector capable of expressing said receptor polypeptide, wherein said vector is selected from RS-hRXR-alpha, RS-mRXR-alpha, or RS-mRXR-gamma and a reporter vector selected from delta-MTV-TRE_p-CAT, delta-TK-TRE_p-CAT, delta-SV-TRE_p-CAT, delta-MTV-TRE_p-LUC, delta-TK-TRE_p-LUC, or delta-SV-TRE_p-LUC.

20

19. A method according to Claim 16 wherein the cells employed are Drosophila melanogaster Schneider line 2 cells co-transformed with a vector capable of expressing said receptor polypeptide, wherein said vector is selected from A5C-hRXR-alpha, A5C-mRXR-alpha, or A5C-mRXR-gamma, and a reporter vector selected from ADH-TRE_p-CAT, ADH-TRE_p-LUC, TK-TRE_p-CAT, or TK-TRE_p-LUC.

20. A labeled single-stranded nucleic acid sequence, comprising at least 20 contiguous bases in length having substantially the same sequence as any 20 or more contiguous bases selected from:

- (i) bases 2 - 1861, inclusive, of the DNA illustrated in Sequence ID No. 1 [hRXR-α], or
- (ii) bases 20 - 2095, inclusive, of the DNA illustrated in Sequence ID No. 2 [mRXR-α], or

- (iii) bases 15 - 1653, inclusive, of the DNA illustrated in Sequence ID No. 3 [mRXR- γ], or
- (iv) the complement of any one of the sequences according to (i), (ii), or (iii).

5

21. A nucleic acid according to Claim 20 which is labelled with ^{32}P .

22. A method of making a receptor polypeptide, wherein said polypeptide is characterized by:
- (1) being responsive to the presence of retinoid(s) to regulate the transcription of associated gene(s); and
- (2) having a DNA binding domain of about 66 amino acids with 9 Cys residues, wherein said DNA binding domain has:
- (a) less than about 65 % amino acid identity with the DNA binding domain of hRAR-alpha,
- (b) less than about 55 % amino acid identity with the DNA binding domain of hTR-beta, and
- (c) less than about 55 % amino acid identity with the DNA binding domain of hGR;
- 25 said method comprising culturing cells containing an expression vector operable in said cells to express a DNA sequence encoding said polypeptide.

23. A method according to Claim 22 wherein said receptor polypeptide has substantially the same sequence as that of:

amino acids 1 - 462 shown in Sequence ID No. 2 [hRXR- α],

35 amino acids 1 - 467 shown in Sequence ID N . 4 [mRXR- α], r

amino acids 1 - 463 shown in Sequence ID No. 6
[mRXR- γ].

24. A method according to Claim 22 wherein said
5 receptor polypeptide comprises a DNA binding domain with
substantially the same sequence as that of:

amino acids 135 - 200 shown in Sequence ID No. 2
[hRXR- α],

amino acids 140 - 205 shown in Sequence ID No. 4
10 [mRXR- α], or

amino acids 139 - 204 shown in Sequence ID No. 6
[mRXR- γ].

25. A method according to Claim 22 wherein said DNA
15 sequence comprises a segment with substantially the same
nucleotide sequence as that of:

nucleotides 76 - 1464 shown in Sequence ID No. 1
[hRXR- α],

nucleotides 181 - 1581 shown in Sequence ID No. 2
20 [mRXR- α], or

nucleotides 123 - 1514 shown in Sequence ID No. 3
[mRXR- γ].

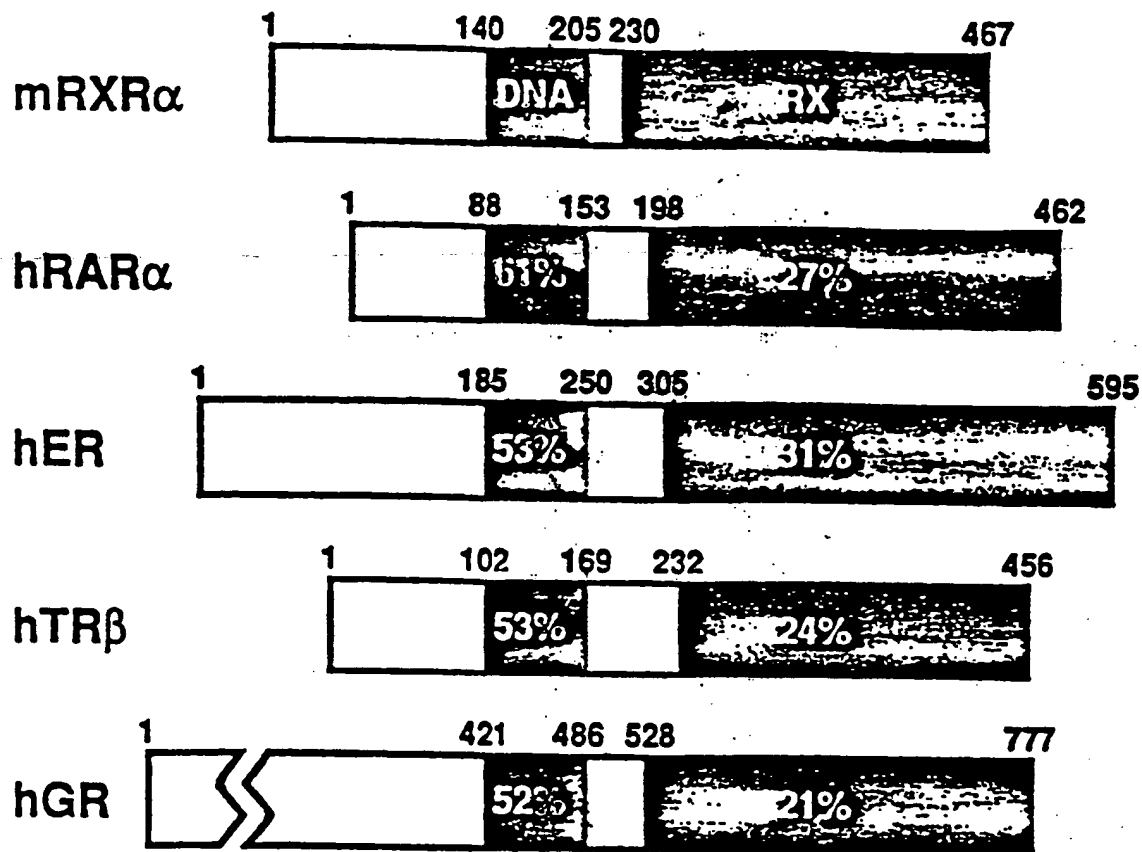
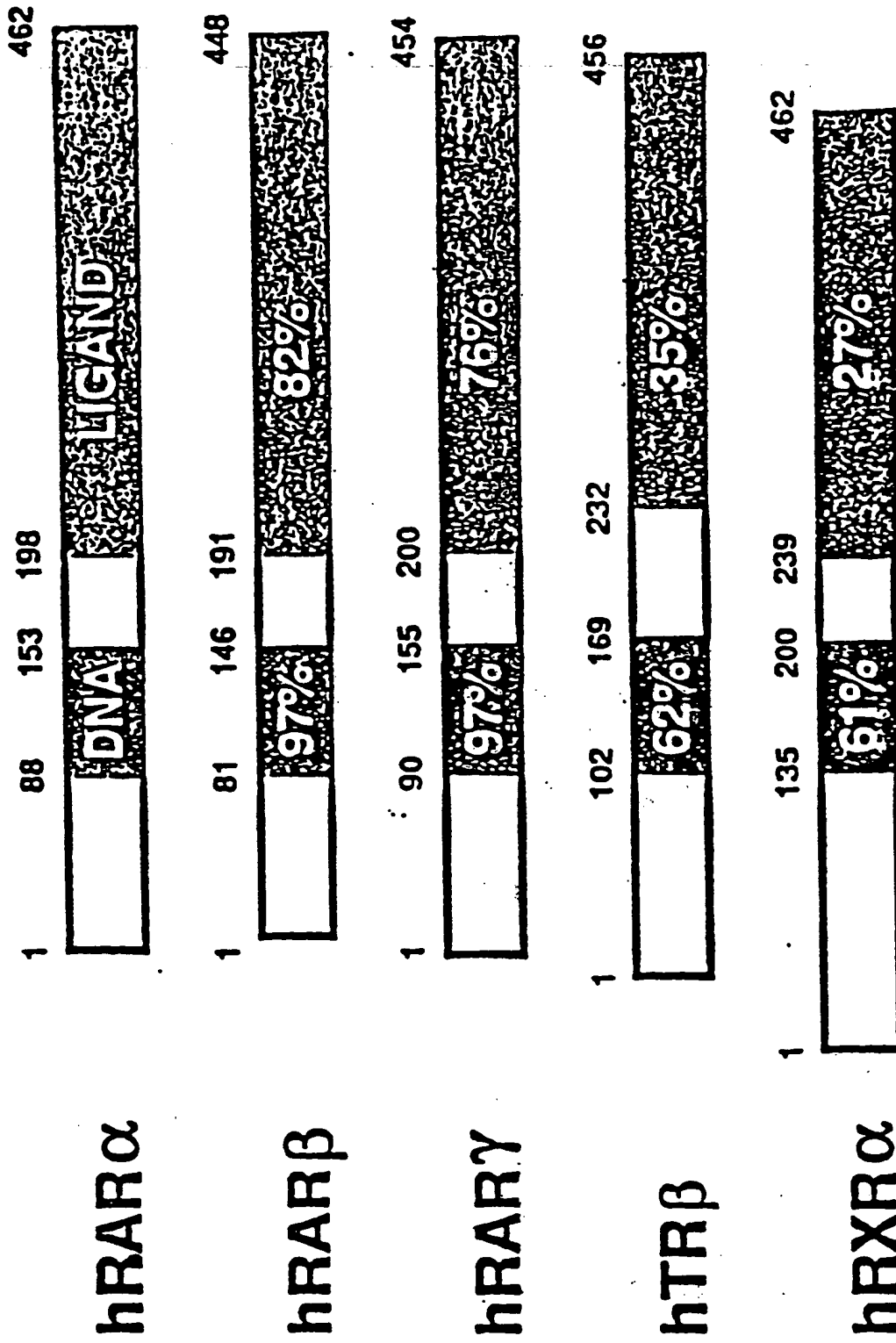


Fig. 1

FIGURE 2



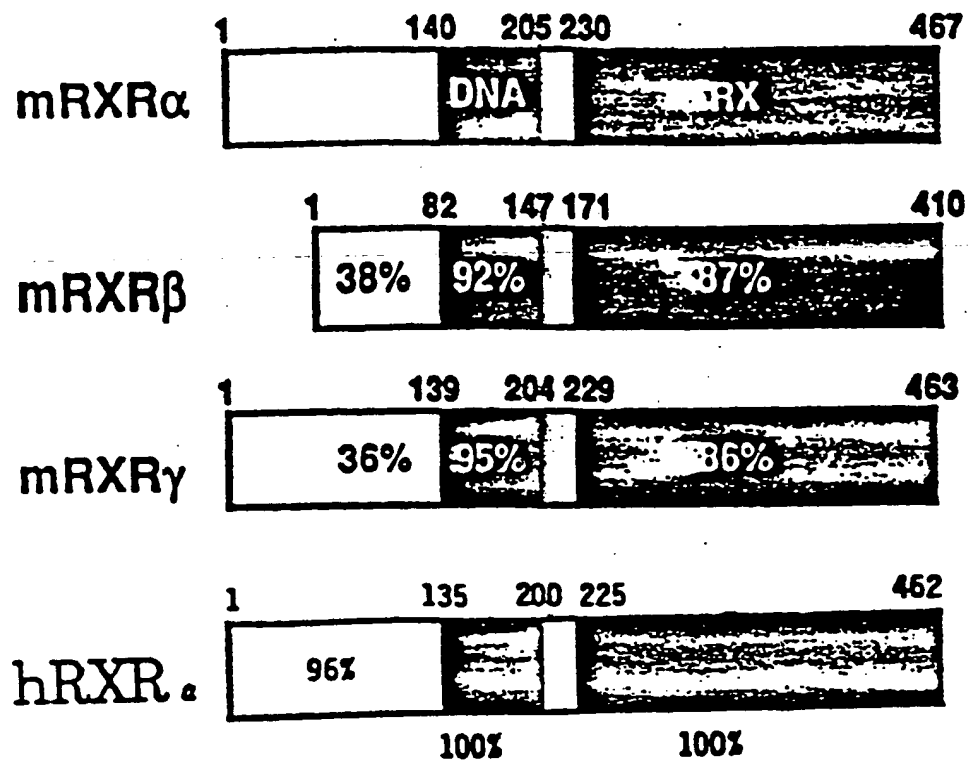


FIG. 3

FIGURE 4

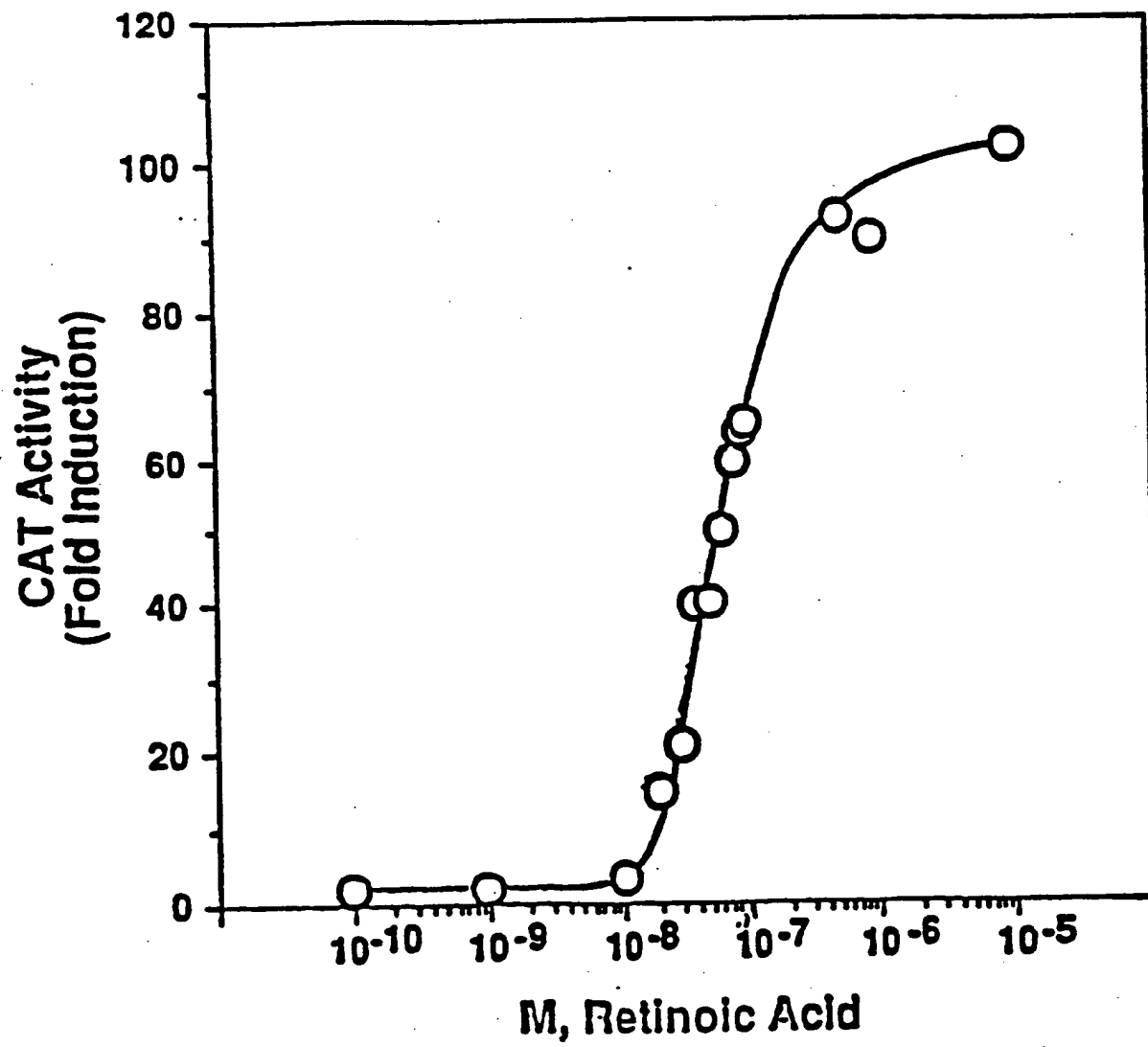


FIGURE 5

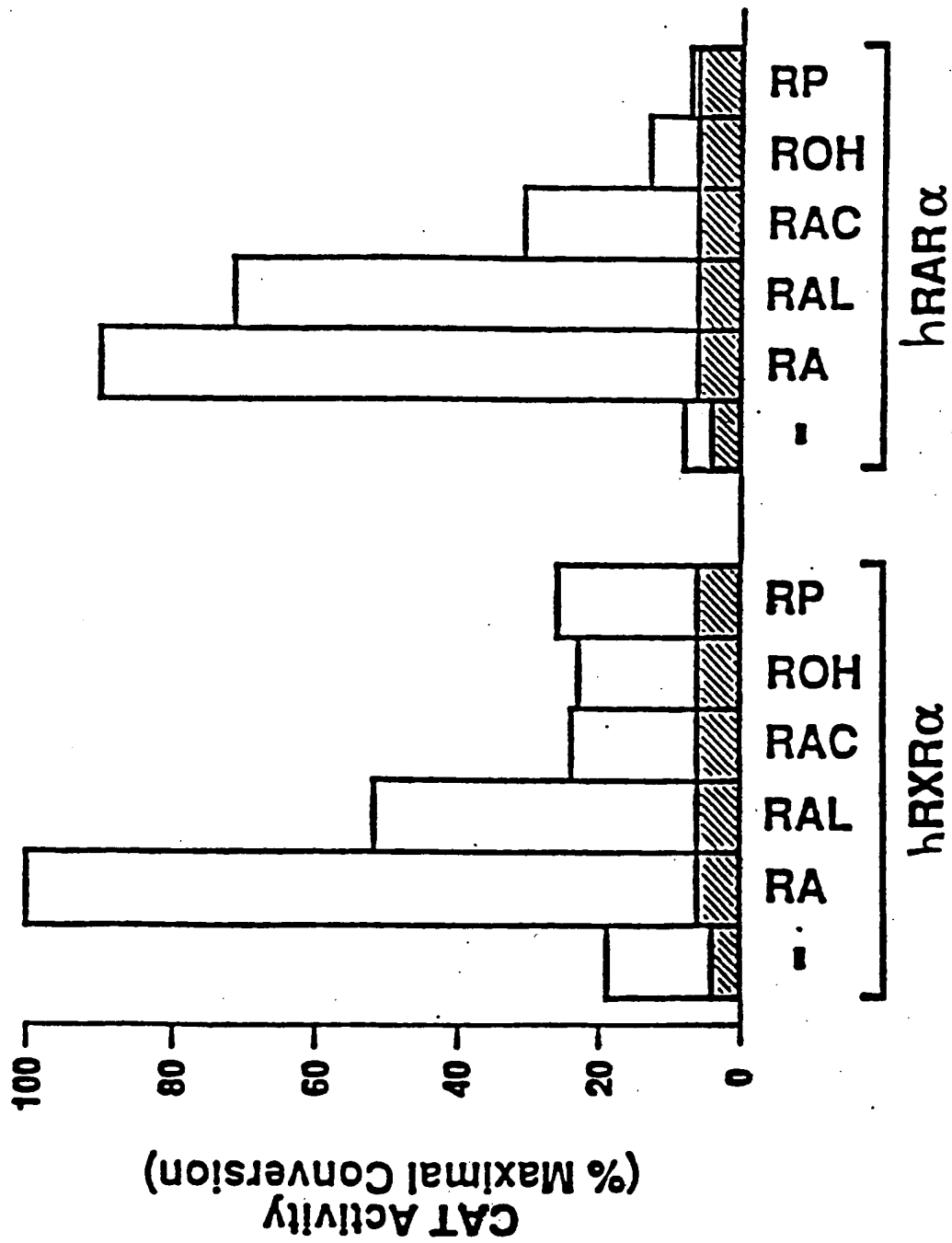


FIGURE 6

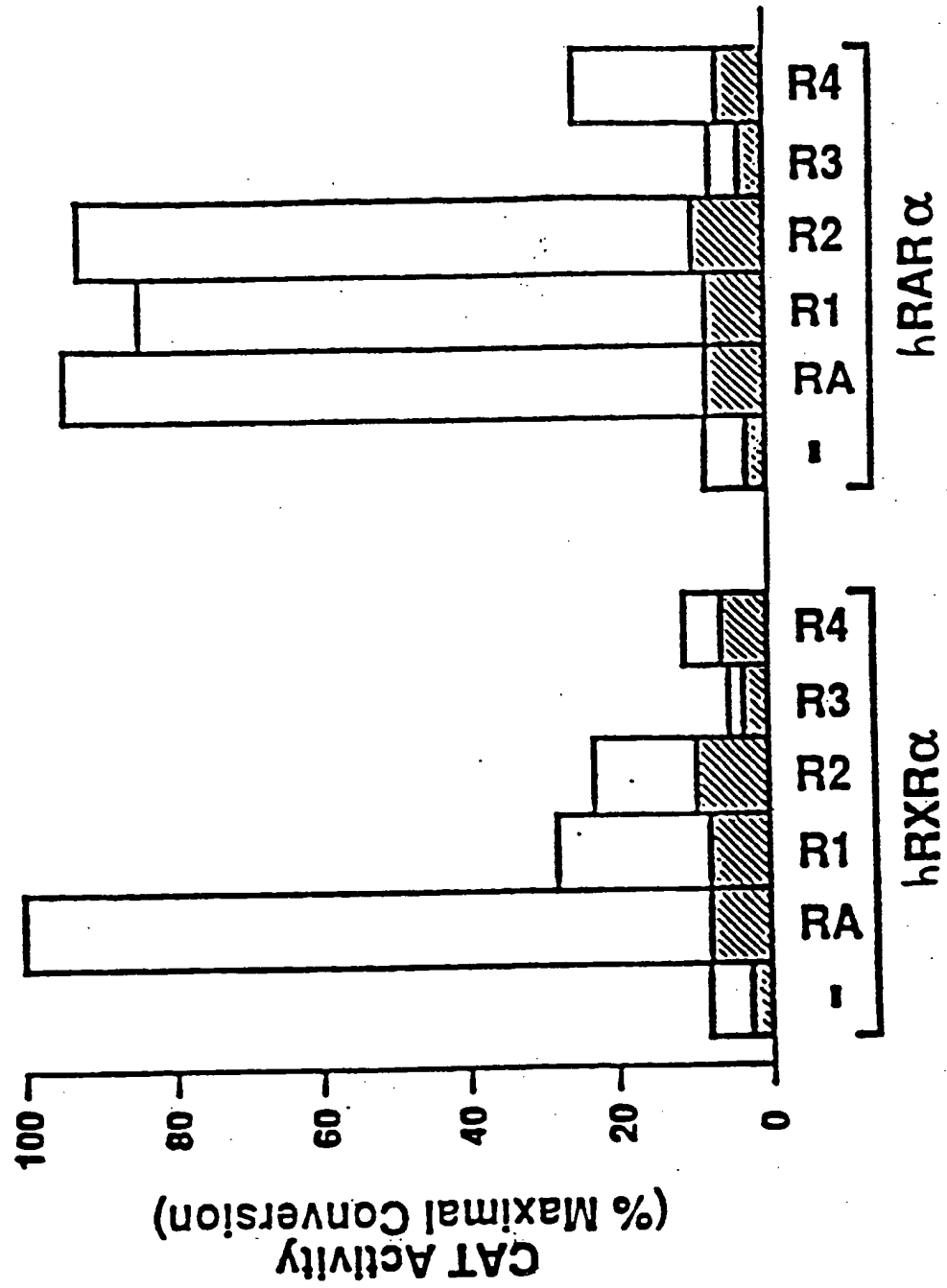
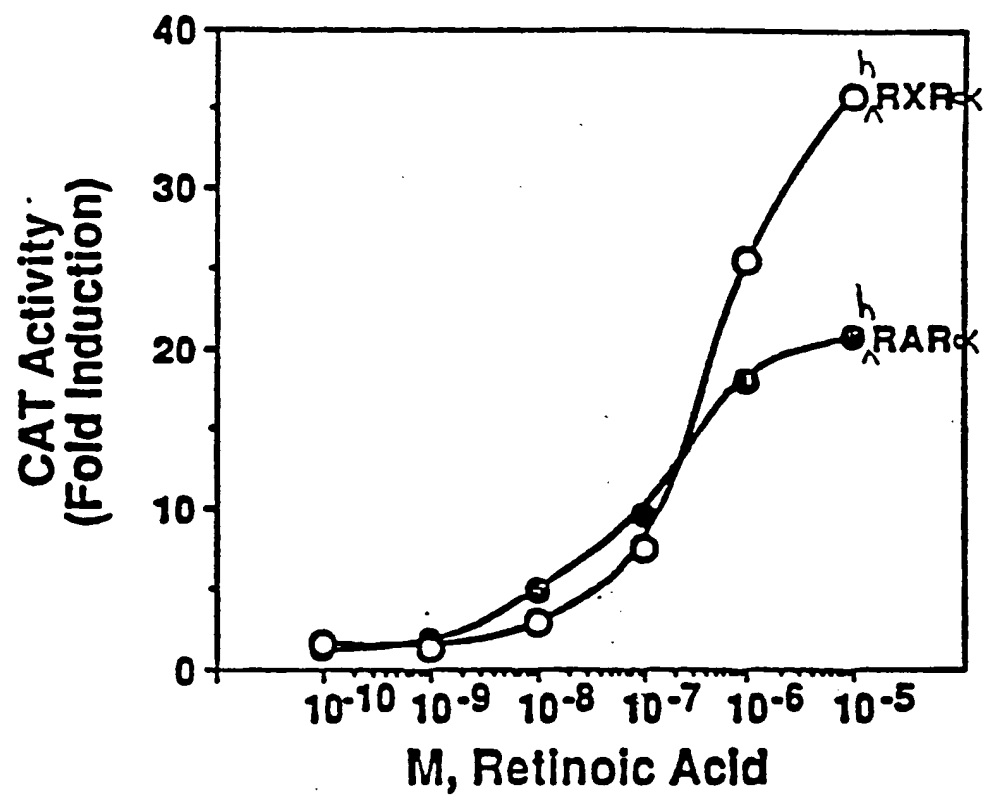


FIGURE 7



a

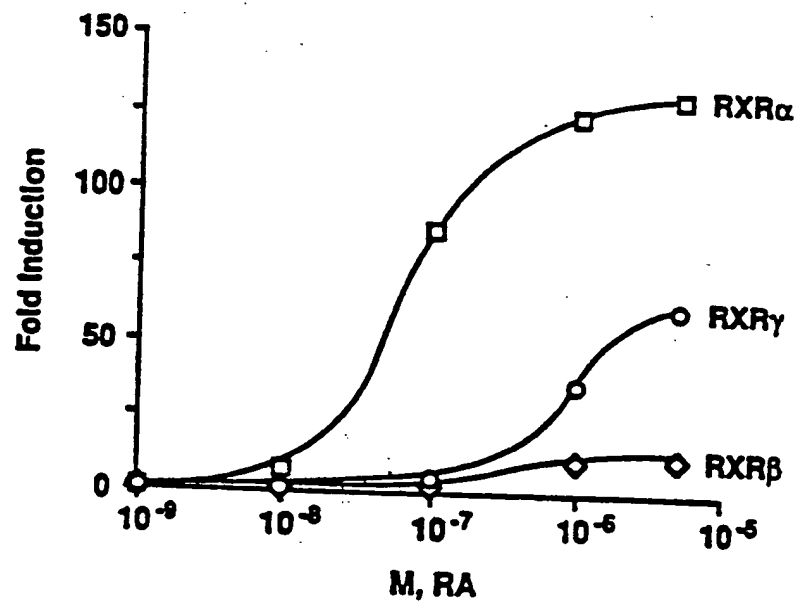


Fig. 8

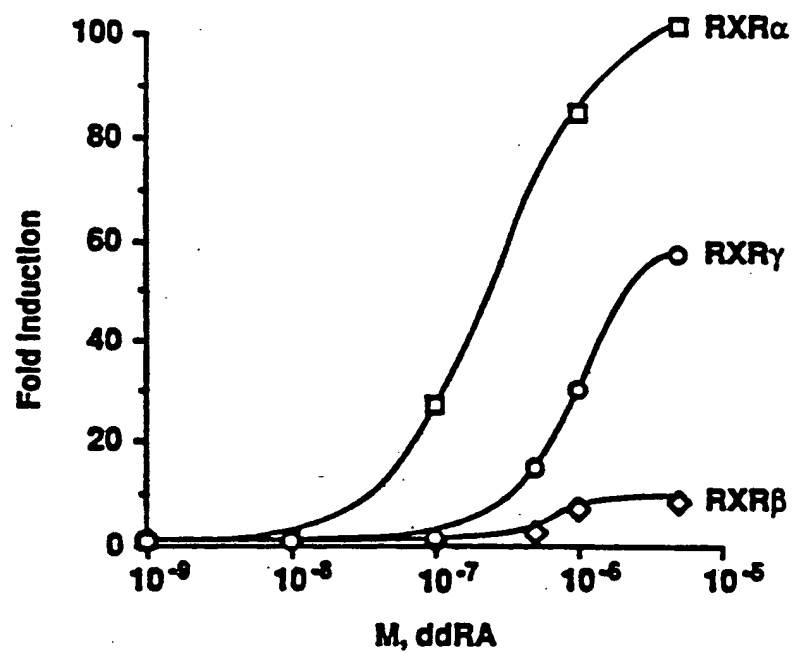
b

Fig. 9

INTERNATIONAL SEARCH REP RT

International Application No. PCT/US91/00300

I. CLASSIFICATION OF SUBJECT MATTER

According to International Patent Classification (IPC) or to both National Classification and IPC
 I.P.C.(5): C07H 15/12; C07K 3/00; C12O 1/68; C12N 15/00
 U.S. Cl.: 536/27; 530/350; 435/6; 935/77.75

II. FIELDS SEARCHED

Minimum Documentation Searched

Classification System

Classification Symbols

U.S. Cl. 536/27; 530/350; 435/6; 935/77.75

Documentation Searched other than Minimum Documentation
 to the extent that such Documents are included in the Fields Searched

Gen Bank, EMBL

III. DOCUMENTS CONSIDERED TO BE RELEVANT

Category * 1 Citation of Document, ** with indication, where appropriate, of the relevant passages 12 Relevant to Claim No. 2

- | | | |
|---|---|------|
| X | Nature, Vol. 337, issued 09 February 1989, Giguere et al. "Spatial and Temporal Expression of the Retinoic Acid Receptor in the Regenerating Amphibian Limb", pages 566-569, see especially Figure 1. | 1-25 |
| X | Nature, Vol. 330, issued 17 December 1987, Giguere et al. "Identification of a Receptor for the Morphogen Retinoic Acid", pages 624-629, see especially Figure 1. | 1-25 |
| X | Nature, Vol. 331, issued 07 January 1988, Giguere et al. "Identification of a New Class of Steroid Hormone Receptors", pages 91-94, see especially Figure 1. | 1-25 |

* Special categories of cited documents

- "A" document defining the general state of the art which is not considered to be a prior art document
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** The document published after the international filing date, but before the date of publication of the international search report, is not considered to be a prior art document for the purposes of the international search report.

IV. CERTIFICATION

Date of the Actual Completion of the International Search

Date of Mailing of the International Search Report

14 March 1991

03 MAY 1991

International Searcher

ISA/US

Mindy B. Fleisher